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Archives of Internal Medicine

VOLUME 49 JANUARY, 1932 NUMBER 1

USUAL AND UNUSUAL REACTIONS TO PROTEIN (FEVER) THERAPY *

PHILIP S. HENCH, M.D.

ROCHESTER, MINN.

Shortly after Wright and others introduced specific vaccine therapy about twenty years ago, nonspecific vaccine therapy was introduced. This was soon followed by nonspecific protein therapy. Typhoid vaccine was first used intravenously by Kraus and Mazza [1] in Argentina about 1912. Its use as a preventive of typhoid fever was first advocated by Ichikawa [2] (1912), and it was first given intravenously as a nonspecific protein for "fever" therapy by Miller and Lusk [3] in 1916 in the treatment for chronic infectious arthritis. It has since become one of the most widely used agents for protein or "fever" therapy, is employed for many diseases, particularly of the joints, skin and eyes, and as a substitute for malarial treatment in late syphilis. Recently it has been successfully used in the treatment for occlusive vascular disease [4] and for evaluating, by means of the "vasomotor index" of Brown, the possibilities of sympathetic ganglionectomy for arthritis [5] and certain vascular conditions.[6]

* Submitted for publication, March 2, 1931.

* From the Division of Medicine, the Mayo Clinic.

* Read before the Section on Pharmacology and Therapeutics at the Eighty-First Annual Session of the American Medical Association, Detroit, June 27, 1930.

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TYPE OF VACCINE AND DOSAGE USED

Many thousands of injections of typhoid vaccine have been given at the Mayo Clinic for the treatment in various conditions, generally supplementary to other treatment. For this report a special group of patients on the arthritis service and on the vascular service of Dr. George Brown was studied in the course of the last five years, the number of which was about 2,500. Of these, approximately 1,500 had arthritis; the remainder suffered from various conditions, chiefly vascular diseases. The number of reactions studied was about 10,000.

Various factors influence the reaction: the kind and degree of the patient's illness; the method of administration, type and freshness of the vaccine; the size of the dose, and the response of the patient. Reactions to typhoid vaccine are basically the same as reactions to other foreign proteins. When the substance is given intramuscularly, reactions are minimized and delayed. When it is given intravenously, reactions are sharper, more prompt. The ability to produce fever and other phases of the reaction vary also with different preparations of vaccine. Fresh vaccine gives a more prompt reaction than old vaccine and should be used in smaller doses. Preparations of milk or sulphur are used less frequently at the clinic. Typhoid vaccines made by state boards of health, the United States Army and commercial houses have been used. On the arthritic and vascular services one commercial preparation has been used more than others because of familiarity with the type of reaction from a given dose. This brand is one of the so-called triple-typhoid preparations made from Eberthella typhi, Salmonella paratyphi and Salmonella schotmuelleri or, in more familiar terms, typhoid and paratyphoid A and B. It probably has no particular merit or demerit, however. It is generally given intravenously; occasionally, intramuscularly. No set schedule of time or dosage is used. Some advise the administration of foreign protein not oftener than once a week; others (Cowie), every second day. When well borne, at the clinic it is given approximately twice a week. The initial dose is usually from 15,000,000 to 25,000,000 bacilli; subsequent doses are 50,000,000, 75,000,000 or 100,000,000, and so on.[7]

Experience suggests that injections in cases of arthritis usually should be discontinued after from six to ten doses; this does not necessarily hold when patients with vascular disease are being treated. There should be an intermission of several weeks to prevent marked loss of weight ("proteaginous cachexia"), to permit the return of normal equilibrium and restoration of the patient's morale, and because

7. The estimation of the number of bacilli in each dose is, of course, only approximate. Although it would be more accurate to state dosage in terms of cubic centimeters of bacterial suspension, the doses are given as they are for comparison, and in line with the custom of the majority of previous writers.

further injections frequently prove ineffective. In arthritis, when three or four intravenously administered doses, with moderate reactions, fail to produce definite relief, or if such doses produce increased disability of the joints, the treatment is discontinued in favor of more conservative (intramuscular) protein therapy or treatment with stock vaccines.

THE USUAL REACTION

Although reactions to the intravenous use of typhoid vaccine and other foreign proteins may at times seem severe to the patient, they appear relatively simple to the physician; they usually consist merely of the common clinical phenomena of chill, fever, mild headache, general malaise, mild gastro-intestinal upset, and so on. Further analysis of the reaction, however, shows more than just an "ordeal by chill and

TABLE 1.—*The More Obvious Reaction to Protein Therapy*

	Prodromal Phase Including (a) Latent or Prechill and (b) Chill Period	First "Negative" Phase to Height of Fever, 3 to 5 Hr. After Injection	Second "Positive" Phase; Period of Falling Temperature Variable, 6 to 24 Hr. After Injection	Subsequent Day
Temperature.....	No increase	Rising	Falling	Occasionally a secondary rise
Chill.....	Minor or distinct	Generally absent	.....	Occasionally a secondary chill
Pain; inflammation; swelling; disability; nervous irritability	Usually absent; may be slight	Increasing	Decreased or absent; euphoria	Variable duration of euphoria

fire," and that marked disturbances in the physical and chemical processes of the body are included.

The general nature of the usual reactions has been extensively studied. It manifests itself as a prodromal phase and in two further phases a "negative" and a "positive" phase, generally considered to be distinct (table 1). The period just after the injection is given, and before the temperature begins to rise, is the prodromal phase, consisting of what may be called a prechill and a chill period. Then there begins the first or negative phase, that period from the onset of the rising temperature to the point of maximal temperature. The second or positive phase extends from the height of the fever to the return of normal temperature. Occasionally a secondary chill and fever occur the following day. During the negative or first phase there are inaugurated certain phenomena, some of which seem deleterious to the patient's well-being and which, if long continued, would militate against the usefulness of the treatment. There is in general, however, a sharp reversal of these phenomena at the beginning of the positive or second phase, and during this reversal the processes of healing are markedly

stimulated. Innumerable attempts have been made to eliminate or shorten the negative phase by using various vaccines, different proteins or protein fractions and different methods of administration. It is generally believed, however, that to obtain a therapeutically positive second phase, there must be an appreciable first phase, and that without the lytic phenomena of the first phase, the healing process which results from stimulation of the defensive mechanism of the body will not be adequate (Donath,[8] Garrison,[9] Maliwa,[10] Miller [11]).

The clinical alterations noted in table 1 constitute the more obvious part of the reaction to foreign protein. Further analysis of the reaction in patients and in animals reveals innumerable marked physical and chemical alterations that demonstrate the intensity of the activation of cellular and humoral defenses. Complete details concerning alterations in the organic activity of all bodily structures cannot be given here. The changes in organic activity are, in general, initiated about the time of the period of chill, and are altered in one direction (increased or decreased) during the first or negative phase, and in the opposite direction during the second or positive phase. The reported changes consist of the following: (1) alterations in the basal metabolism; (2) peripheral and splanchnic vasomotor changes, including changes in blood pressure and in the caliber and permeability of the arterioles and capillaries; (3) alterations in renal function detectable in the output and acidity of the urine, excretion of phenolsulphonphthalein, and concentration of nitrogen, phosphate, urea, uric acid, allantoin and albumin; (4) alterations in serum ferments, antiferments, antibodies and the Wassermann reaction; (5) alterations in organic activity demonstrated by increased secretion of lymph, bile, saliva, breast milk and menstrual flow, and by changes in the activity of the liver, gastro-intestinal tract and spleen; (6) alterations in the volume, specific gravity, freezing point and viscosity of the blood; (7) alterations in the cellular elements of the blood; (8) alterations in the fragility of the blood platelets and in fibrinogen, thrombokinase, coagulation time and sedimentation rate; (9) alterations in the chemical constituents of the blood, demonstrated by the carbon dioxide tension of the plasma, carbon dioxide-combining power, total nonprotein nitrogen of whole blood and of

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serum, sugar tolerance, albumin-globulin ratio and concentration of urea, uric acid, sugar, fat, total serum protein and chlorides.

Some of the alterations are very slight, temporary and perhaps clinically insignificant. Others are marked and significant from the standpoint of both usual and unusual reactions, and to them are ascribed the successes, negative results and also, probably, the mishaps of protein therapy. The increasing use of protein therapy attests to the belief that although negative results are common, satisfactory, even brilliant, results are many times obtained and mishaps are rare.

Experimental Data.—The amazing complexity of the reaction could be more simply understood if it were demonstrated that all changes were initiated through a central mechanism. That the changes are initiated through the sympathetic nervous system was suggested, among others, by Vaughan [12] and by Gellhorn.[13] Petersen and Müller [14] and Petersen, Müller and Boikan [15] have made a most interesting effort to correlate the various alterations and to demonstrate their coordinated activity. They concluded that when live or dead bacteria, or proteins, are injected intravenously, the injected material is removed from the blood stream by the cells of the reticulo-endothelial system and there "fixed." There is first a fixation (prolonged stimulation) of the splanchnic organs (liver, gastro-intestinal tract and spleen especially) in a state of increased organic activity, and of the splanchnic blood vessels in a state of dilatation. At the same time there is a compensatory, opposite orientation or "fixation" in the activity of peripheral organs and vessels. This is demonstrated by depression in the metabolic activity of skin and muscles and constriction in the peripheral blood vessels. Complete reversal of this state later occurs in both the splanchnic and the peripheral regions, and then there is a return to normal equilibrium. If the injections are given continuously, or if they are repeated beyond the point of tolerance, the repeated stimulation leads to the death of cells.

If the reaction in human beings is similarly motivated and expressed, particularly the vascular and vasomotor changes, certain features of the unusual reactions here reported seem more explicable.

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THE UNUSUAL REACTION

When one considers the profound response to an injection, unusual and untoward reactions might be expected not infrequently; yet they are rare. Large series are reported with no untoward response, even to high doses. At the clinic in this series of about 10,000 injections in 2,500 cases, unusual clinical phenomena have been observed subsequent to only twenty injections and in only fourteen cases, but in view of the increased usage of protein therapy they seem of sufficient importance to report. It would be unfair to ascribe to vaccine treatment responsibility for every clinical alteration that occurred in the course of its administration. Therefore, I have here reported only those unusual reactions which seemed to be related to the protein or fever therapy by reason of their unexpectedness, the promptness of their onset after an injection, the nature of their clinical expression and their similarity to previous experiences of others. The reports of unusual reactions observed by others have not heretofore been reviewed extensively, are widely scattered and are given with but brief comments.

I shall present, in briefest detail, data concerning reactions which these fourteen patients experienced within a half hour to forty-eight hours after the injection, and I shall briefly comment on the possible causal relationship between the injection and the reaction. I was not looking for these reactions, and lacked appreciation of their significance until their repetition stimulated particular observation as they occurred and an extensive review of the literature.

The cases are not presented in historical sequence, but are grouped according to the reaction.

CASE 1.[16]—A woman, aged 53, had chronic infectious arthritis. Previous to the administration of typhoid vaccine her heart and lungs appeared to be normal. After the first dose of typhoid vaccine (35,000,000 bacilli), there was a severe chill but no fever. The increase in temperature began the next morning, and rose to 103 F.; the following day it rose to 104 F. A precordial murmur appeared, and for four days the fever was of the septic type. Râles were present at the bases of both lungs, especially the left. Roentgenograms gave evidence of a thickened pleura at the base of the left lung, and some fluid. Eight days after the injection the temperature and pulse rate returned to normal and remained normal. On dismissal the precordial rub was diminished; dulness at the base of the left lung persisted. The condition was supposed to have been subacute pleurisy and pericarditis. Eighteen months later, examination of the lungs and heart gave objectively negative evidence.

CASE 2.[16]—A woman, aged 51, had chronic infectious arthritis. The lungs were normal on examination. The first two injections (25,000,000 and 35,000,000 bacilli, respectively) were followed by moderate reactions, a temperature of 103 F. and diminution of pains in the joints. Twelve hours after the third injection (35,000,000 bacilli), while fever was present, a sore throat developed, and the following day there was sharp pain at the base of the left lung on breathing. The tonsils had

16. The recollection of two previous additional cases in which pleurisy and definite pericarditis had occurred immediately subsequent to the injection of vaccine stimulated these special observations.

been removed. Fever continued for nine days; the symptoms and signs were those of subacute basal pleurisy and pharyngitis. Recovery followed.

CASE 3.—A man, aged 32, had chronic infectious arthritis. He had been exposed to contagious parotitis two weeks previous to his coming to the clinic, but he had this disease at the age of 22. Following the first four injections (from 15,000,000 to 50,000,000 bacilli) there were marked reactions and definite improvement in the condition of the joints. A few hours after the fifth injection (50,000,000 bacilli) acute soreness of the left parotid region developed. The gland became about 3.5 cm. in diameter; there were definite induration and tenderness graded 2, which subsided entirely by the fifth day.

CASE 4.—When the patient was 18 years of age, a node in the right groin had enlarged and become suppurative and it had been drained. It had been normal since that time. She was 37 years of age when I saw her. After each of five injections (30,000,000, 40,000,000, 40,000,000, 50,000,000 and 100,000,000 bacilli) there was a moderate general reaction and definite improvement in the condition of the joints, but there was painful swelling of the right inguinal nodes, which did not recur after the cessation of treatment. Six months later there had been no further adenitis, and the condition of the joints was markedly improved.

CASE 5.—A man, aged 55, gave no history of ocular trouble. He had chronic infectious arthritis. The vision of each eye was recorded as 6/15. The pupils and reflexes were normal. The patient read well with a plus 3 lens. Exophthalmos measured 22 mm. on the right and 21 on the left. Refraction was advised. After the second injection (50,000,000 bacilli) high fever and chills occurred. The next morning there were pain and photophobia in both eyes, with conjunctivitis. The following day the eyes were very painful and acutely inflamed; the cornea of each was hazy; there were congestion of the ocular conjunctiva and definite bilateral inflammatory glaucoma. Tension on palpation was graded + 1 in the right eye and + 2 in the left. There was no ciliary injection or iritis. Under treatment, improvement resulted, and the eyes were normal four weeks after the injection. Seven months later there was no ocular trouble.

CASE 6.—A man, aged 53, who had chronic infectious arthritis, had had twelve attacks of gallbladder colic over a period of forty years, but none for five years. There was no abdominal tenderness, but roentgen examination of the gallbladder indicated cholecystic disease. The patient worked in a mine and had had a chronic cough for twenty years, the cause of which, after roentgen examination, was said to be pneumonoconiosis. Examination of the lungs disclosed only some emphysema and harsh breath sounds. After the initial dose of typhoid vaccine (20,000,000 bacilli) there was a moderate reaction; the temperature was 103 F., but became normal in twelve hours. Just after the chill, however, pain similar to the old gallbladder attacks developed, and there was definite tenderness in the right upper abdominal quadrant apparently due to subacute cholecystitis. This continued for two or three days, with a return of fever to 102 F. Ten days later, without more vaccine, subacute asthmatic bronchitis developed which continued a chronic course for two months. One year later it was reported that the patient had not had symptoms of pulmonary trouble since.

CASE 7.—Since appendectomy six years previously, a woman who had chronic infectious arthritis had had mild soreness in the right upper abdominal quadrant four or five times a year. Five months previous to admission she had noted the onset of fever and diarrhea with from eight to ten stools a day. For the last three months she had had mild, weekly attacks of soreness in the right upper abdominal quadrant. The diagnosis, made elsewhere, was possibly typhoid fever, with typhoid cholecystitis, and three doses of typhoid vaccine had been given intravenously.

without influence on the abdominal symptoms. The Widal test was negative. The patient was 29 years of age when she came to the clinic. Roentgenograms of the gallbladder at the clinic showed normal excretion of dye but "a small stone or papilloma in the fundus." Half an hour after the first injection there had been a sudden onset of soreness over the region of the gallbladder, which lasted six hours. The right side of the abdomen was tender. Two hours after injection, the gums, at the site where teeth had been extracted four days previously, suddenly became sore. The pain lasted half an hour. There was considerable focal reaction in the joints. On the following day the temperature was 100.5 F. Three days later the second injection (50,000,000 bacilli) was given. There was a moderate, generalized reaction. Two hours after injection there was a sudden recurrence of the pain in the right upper abdominal quadrant. Soreness and tenderness lasted all night, associated with a focal exacerbation in the joints. There was no soreness at the site of the recent dental extraction, but a sudden onset of toothache in the region of a condemned tooth. Operation was advised. Chronic catarrhal cholecystitis, with a papilloma at the fundus of the gallbladder, was found. Typhoid bacilli could not be cultured from the gallbladder. Recovery was uneventful.

CASE 8.—For several summers a woman, aged 28 when she came to the clinic, had had short attacks characteristic of acute appendicitis. The last attack had occurred one year previous to admission to the clinic. On admission, for chronic infectious arthritis, there was definite tenderness in the region of McBurney's point. The patient would not consider operation. There was very little systemic reaction to the first three injections of vaccine (25,000,000, 35,000,000 and 35,000,000 bacilli, respectively). After the fourth injection (50,000,000 bacilli) the temperature was 101 F. On the following morning the temperature was also 101 F., and there were chills and vomiting. In the afternoon there was localized abdominal soreness. Operation was performed and appendicitis without peritonitis was found. The report on examination of the removed tissue was that of acute hemorrhagic appendicitis. Recovery was uneventful.

CASE 9.—Three months previous to admission for chronic infectious arthritis, a man, aged 40, had had an acute attack of disease of the gallbladder. The gallbladder and appendix were removed. After the first injection (25,000,000 bacilli) there was a slight reaction and the temperature rose to 100 F. Then it became normal. Thirty-six hours after injection the temperature suddenly rose to 101 F. There were: severe, general abdominal pain, nausea and vomiting, tenderness over the scar of the operation on the gallbladder, and diarrhea. Abdominal distress continued for three days; then it completely ceased. Apparently the condition was subacute enteritis. Two years later it was reported that there had been no abdominal discomfort or diarrhea since the patient's dismissal.

CASE 10.—A woman, aged 50, had rheumatic fever which was unresponsive to ordinary treatment; she also had erythema nodosum. She gave no history of previous abdominal trouble. After the first injection (15,000,000 bacilli) there was a febrile reaction and the condition of the joints was much improved. The erythema nodosum commenced to fade. The temperature was occasionally normal throughout the next four days. After the second injection (15,000,000 bacilli) there was a moderate reaction. The following morning the patient felt very well. In the afternoon there was a sudden onset of nausea and vomiting; general abdominal tenderness appeared and became localized in the right lower quadrant. The leukocytes numbered 14,800 per cubic millimeter of blood. Operation disclosed acute diffuse purulent appendicitis and periappendicitis. Recovery was uneventful. One year later the patient was in excellent health.

CASE 11.—A woman, aged 59, had arteriosclerotic vascular occlusion affecting the feet. She had had corneal trouble, but it had been inactive for several years.

The first two injections (15,000,000 and 25,000,000 bacilli, respectively) were well borne. The third injection (35,000,000 bacilli) caused right palpebral conjunctivitis. There was no definite iritis. The fourth injection (25,000,000 bacilli) was well borne. After the fifth injection (50,000,000 bacilli) there was a moderate reaction. On the following day, redness of the right eye was seen. This developed into typical dendritic keratitis of the herpetic type. Subsequently, this ceased. After three weeks there was complete healing.

CASE 12.—A man, aged 52, had had pains of claudication in the right leg for one year. The femoral and popliteal arteries were pulsating. The large arteries of the feet were occluded. The urine was normal on examination. The concentration of urea in the blood was 34 mg. per hundred cubic centimeters. The patient was admitted to the hospital in order that the vasomotor index might be determined. One injection (50,000,000 bacilli) was given. One hour later chills appeared and lasted for twenty-five minutes. That afternoon there was a sudden onset of severe pain from the back of the left leg to the left lumbar region and numbness of the left foot. The femoral pulse was lost. The pain was excruciating and was not controlled by opiates. On the following morning, anuria was present. That night the patient died in spite of efforts to restore renal function. At postmortem examination, advanced arteriosclerosis and thrombosis of the iliac arteries were found. The kidneys were essentially normal.

CASE 13.—A man, aged 51, had undergone resection of a malignant papilloma of the stomach five months previous to admission. Recently he had had acute polyarthritis. The urine was normal on examination; the recovery of phenolsulphonphthalein was normal. The concentration of urea in the blood was 17 mg. per hundred cubic centimeters. The history was negative for lower intestinal or renal disability. Because of severe chronic infectious arthritis, which was not relieved by ordinary measures, one injection (25,000,000 bacilli) was given. There was a moderate reaction, with a rise in temperature to 100 F.; then the temperature became normal. On the following morning there was a secondary rise in temperature. Diarrhea appeared, with diffuse abdominal soreness and slight rigidity, which continued the following day. There was no localization of the abdominal pain. The leukocytes numbered 30,000 per cubic millimeter of blood. The third day after the vaccine had been given, anuria and cessation of diarrhea developed. The concentration of urea in the blood was 116 mg. per hundred cubic centimeters. Anuria continued and the concentration of urea in the blood rose to 270 mg. per hundred cubic centimeters in spite of repeated intravenous injection of diuretics and the use of hot packs. Bilateral renal capsulotomy and left renal sympathectomy were performed. The following four days, the output of urine increased to 500 cc. a day, but the concentration of urea in the blood rose to 236 mg. per hundred cubic centimeters. Coma and death supervened. At postmortem examination evidence of acute nephritis or definite arteriosclerotic changes was not found. The anuria was unexplained on the basis of the condition of the renal tissue. Acute ulcerative colitis, acute purulent prostatitis, arteriosclerosis graded 2 and subacute pericarditis were found.

CASE 14.—A man, aged 38, presented marked deformities, generalized emaciation and secondary anemia. He had chronic infectious arthritis. Transfusion was performed. The urine was essentially normal on examination. Keratitis developed. Protein therapy was suggested. After the first injection (50,000,000 bacilli) a moderate reaction appeared. Pains in the joints improved. Ten hours later, a sore throat developed. On the following day the temperature was 103 F., and pharyngitis, laryngitis and edema of the epiglottis were present. One week after the injec-

tion the condition of the eye was improving, although that of the throat was not. In the urine, the amount of albumin was graded 1, and the number of erythrocytes, 3. Sixteen days after the injection slight bronchitis was present, and twenty-six days after the injection anuria appeared. Anuria continued for three days in spite of treatment. Cardiac failure and death supervened. At postmortem examination, bronchitis, bronchiectasis, abscess and slight empyema were found. Acute diffuse nephritis also was present, but there was no evidence of previous nephritis or of definite arteriosclerotic changes in the kidneys.

Reports by Others.—Although the reactions recounted here were diverse, they were, including fatalities, in general comparable to those which have occurred elsewhere after the use of various proteins and which have been reported by a number of clinicians. The reactions that have been reported represent a very small incidence among the enormous number of patients who must have been treated by means of foreign proteins. Particularly noteworthy are the cases reported by Irons,[17] Miller,[18] Schmidt,[19] Petersen,[20] Ginsburg,[21] Allen and Smithwick [22] and Richardson.[23]

Reactions in arthritic patients after the intravenous administration of typhoid vaccine have been reported as follows: delirium tremens, pneumonia and death of a patient with alcoholism and arthritis (Miller, Scully [24]); delirium tremens and recovery in four additional such cases (Cecil,[25] Miller, Scully); delirium and recovery of a patient with arthritis who did not have alcoholism (Cowie and Calhoun [26]); vomit-

17. Irons, E. E.: The Treatment of Gonococcus Arthritis by Injections of Dead Gonococci, and the Clinical Reaction which Follows the Injections, Arch. Int. Med. **1**:443 (May) 1908; The Clinical Use of Foreign Protein, Tr. A. Am. Physicians **32**:91, 1917.

18. Miller (footnote 10, first and second references).

19. Schmidt, Rudolf: Zur Frage der Herdreaktionen ihrer Spezifität und ihrer diagnostisch-therapeutischen Bedeutung, Deutsches Arch. f. klin. Med. **131**: 1 (Dec. 12) 1919; Ueber das Problem der Proteinkörpertherapie, Med. Klin. **2**: 695 (July 4) 1920.

20. Petersen, W. F.: Serum Changes Following Protein "Shock" Therapy, Arch. Int. Med. **20**:716 (Nov.) 1917; The Non-Specific Reaction, J. A. M. A. **76**:312 (Jan. 29) 1921; Protein Therapy and Nonspecific Resistance, New York, The Macmillan Company, 1922.

21. Ginsburg, I. S.: Complications in Protein Therapy, Vrach. gaz. **30**:1003 (Oct. 31) 1926.

22. Allen, A. W., and Smithwick, R. H.: The Treatment of Vascular Lesions of the Extremities, J. Michigan M. Soc. **28**:38 (Jan.) 1929; Thrombosis of Peripheral Arteries Following the Intravenous Injection of Typhoid Vaccine: Report of Two Cases, New England J. Med. **200**:217 (Jan. 31) 1929.

23. Richardson, H. E.: Personal communication to the author.

24. Scully, F. J.: The Reaction after Intravenous Injections of Foreign Protein, J. A. M. A. **69**:20 (July 7) 1917.

25. Cecil, R. L.: A Report on Forty Cases of Acute Arthritis Treated by the Intravenous Injection of Foreign Protein, Arch. Int. Med. **20**:951 (Dec.) 1917.

26. Cowie, D. M., and Calhoun, Henrietta: Nonspecific Therapy in Arthritis and Infections, Arch. Int. Med. **23**:69 (Jan.) 1919.

ing, epistaxis and death (Irons); hemiplegia in a case of acute arthritis, with septic endocarditis (Cowie [27]); cardiac dilatation in a case of acute articular rheumatism with mitral endocarditis (Scully); hematuria (Campbell [28]), and frequent herpes labialis (Cecil and others). Thomas [29] stated, also, that he had heard of several deaths in patients with acute arthritis and serious complications, but details of them are not available.

In four cases of arteriosclerotic vascular disease, acute thrombosis has occurred. Allen and Smithwick reported femoral thrombosis and popliteal thrombosis, both necessitating amputation. Richardson had two fatalities following acute femoral thrombosis.

Marked temporary nephritis and exacerbation of cutaneous conditions occurred in dermatologic cases (Engman and McGarry [30]); hemorrhage into the iris, in a case of tuberculous iritis (Cowie); facial herpes in one case of general paralysis, and acute abscess about a symptomless dental root, associated with mania, in another case (Mackenzie [31]).

A group of observers have reported untoward occurrences after typhoid vaccine had been given intravenously in cases of typhoid fever. These have included vasomotor collapse, delirium, psychosis, epistaxis, enteritis and, in eleven cases, death following hemorrhage, usually intestinal (Gay and Chickering,[32] Von Adelung,[33] McWilliams,[34] Kibler and McBride,[35] Fairley,[36] Miller).

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27. Cowie, D. M.: XVIII. Nonspecific Protein Therapy in Arthritis, J. A. M. A. **76**:310 (Jan. 29) 1921.

28. Campbell, A. D.: The Use of Non-Specific Foreign Protein in the Treatment of Inflammatory Lesions in the Female Pelvic Organs, Canad. M. A. J. **16**:413 (April) 1926; Non-Specific Therapy in Rheumatoid Arthritis, Glasgow M. J. **103**:79 (Jan.) 1925.

29. Thomas, H. B.: Arthritis and Foreign Protein, J. A. M. A. **69**:770 (Sept. 8) 1917.

30. Engman, M. F., and McGarry, R. A.: Treatment of Certain Diseases of the Skin, J. A. M. A. **67**:1741 (Dec. 9) 1916.

31. Mackenzie, J. M.: Pyrexia Induced by Intravenous Protein Therapy in General Paralysis, Lancet **2**:223 (July 30) 1927.

32. Gay, F. P., and Chickering, H. T.: Treatment of Typhoid Fever by Intravenous Injections of Polyvalent Sensitized Typhoid Vaccine Sediment, Arch. Int. Med. **17**:303 (Feb.) 1916.

33. Von Adelung, Edward: Vaccine Treatment of Typhoid, California State J. Med. **18**:175 (May) 1920.

34. McWilliams, Helen I.: The Treatment of Typhoid Fever with Typhoid Vaccine Administered Intravenously, M. Rec. **88**:648 (Oct. 16) 1915.

35. Kibler, C. S., and McBride, L. F.: Intravenous Injection of Typhoid Vaccine, J. Infect. Dis. **21**:13 (July) 1927.

36. Fairley, K. D.: The Treatment of Typhoid Fever by Intravenous Vaccines, M. J. Australia **2**:291 (Sept. 22) 1923; A Preliminary Report on the Treatment of Typhoid Fever with Intravenous Vaccines, ibid. **2**:428 (Nov. 12) 1921.

After the intramuscular and subcutaneous use of typhoid vaccine, acute pleurisy in several cases of pulmonary tuberculosis was noted by Clovis and Mills.[37] After its use for vaccination there have been reported cases of acute appendicitis that have developed from latent or quiescent appendicitis, focal pulmonary reactions in tuberculosis, bilateral atrophy of the optic nerve, axillary adenitis (five cases), rheumatic purpura, acute diarrhea (ten cases), asthenia, mania, one death from acute

TABLE 2.—*Unusual Reactions to Protein Therapy (Other Than Typhoid Vaccine) Reported Elsewhere*

Author	Substance Used	Substance Given for	Reaction
Ginsburg [21]	Milk	Arthritis	Acute appendicitis (latent)
Schaverin (quoted by Ginsburg) [21]	Milk	Arthritis	Acute appendicitis (latent)
Irons [17]	Meningococci intravenously	Gonorrheal arthritis	Anuria; death (thrombosis of left renal artery)
Ginsburg [21]	Milk	"Joint disease"	Adenitis; lymphangitis
Sudler (J. A. M. A. **75**: 176 [July 17] 1920)	Phylacogen	Rheumatism	Death ("anaphylaxis")
Schmidt, DeCourcy [54]	Milk	Arthritis	Tonsillitis; exacerbation
Kaess (Deutsche Ztschr. f. Chir. **183**: 316, 1923)	Various	Arthritis	"Bloody diarrhea"; enteritis (?)
Laurie (M. J. Australia **1**: 309, 1923)	Bacillus coli intravenously	Rheumatoid arthritis	Vasomotor collapse; uremia; death
Lamson (J. A. M. A. **82**: 1091 [April 5] 1924)	Antistaphylococcus serum	Acute rheumatism	Death (thrombophlebitis of iliac veins)
Petersen [20]	Various	Arthritis; old cardiac lesion	Cardiac enlargement
McLean (J. A. M. A. **60**: 588 [Feb. 22] 1913)	Phylacogen	Articular rheumatism	Circulatory collapse; anuria; death
Schmidt, DeCourcy	Milk	.....	Acute cholecystitis (latent)
Beigelman (California & West. Med. **30**: 404, 1929)	Milk	Uveitis	Secondary glaucoma
Irons [17]	Meningococci intravenously	Typhoid fever	Edema of lung; hemorrhage of brain and eye; death
Hektoen and Irons [60]	Polyvalent stock vaccine	Thrombophlebitis	Pulmonary embolus
Wood [43]	Phylacogen	Pneumonia	Death ("anaphylaxis")
Wood	Phylacogen	Typhoid fever	Death ("anaphylaxis")
Darier [39]	Milk	Keratitis	Death (cardiovascular depression)
Laufer [71]	Novaprotein	Duodenal ulcer	Severe hemorrhage; cachexia

cardiac dilatation and eight deaths from anaphylaxis. Unusual reactions to proteins other than typhoid vaccine are given in table 2.

In addition, I have found reports written by about thirty different observers of about forty other different reactions occurring after the administration of a variety of substances in a group of patients with diverse complaints. Many were severe focal reactions of the condition primarily under treatment; hence, unusual only in degree. Others were more unusual, appearing in unsuspected sites. They included such reactions as the following: activation of latent and quiescent

37. Clovis, E. E., and Mills, G. E.: Effects of Typhoid Fever and Typhoid Vaccine on Pulmonary Tuberculosis, J. A. M. A. **74**:297 (Jan. 31) 1920.

urticaria, asthma, epilepsy, gout and malaria; provocation of bleeding in cases of hemophilia and epistaxis, and of gastric crises in tabes; painful transient splenomegaly; onset of "cloudy vision" in albuminuric retinitis; hematuria in cases of tuberculous kidney; acute furunculosis in cases of diabetes; exacerbation of pain and swelling in tuberculous lymphoma and gonorrheal bubo; perforation of corneal ulcers; other untoward ocular reactions in cases of disease of the eyes;[38] activation of old quiescent typhoidal spondylitis and tuberculous spondylitis; exacerbation of various cutaneous lesions such as tuberculids, syphilids, leprosy and others; increased secretion in old fistulas and in cases of gonorrheal infection of the genito-urinary tract; increased bronchiectatic excretion, and focal pulmonary reactions in cases of latent tuberculosis.

Sudden deaths generally considered "anaphylactic" have been reported by Darier,[39] Shipp,[40] Russel,[41] Myers,[42] Wood,[43] Thomas,[29] Lemoine,[44] and in the United States Naval Medical Bulletin.[45] It is possible that some of them may have been from acute vasomotor collapse, rather than from true anaphylaxis (Scully [24]).

In considering the types of unusual reactions seen here, in particular appendicitis, cholecystitis, glaucoma, respiratory infections and ulcerative colitis, it must be realized that for these very conditions protein therapy has been used with impunity, and at times even with success. It has been used for appendicitis by Erdély;[46] for primary glaucoma by Patton[47] and by Berneaud;[48] for secondary glaucoma by Howard;[49]

38. Benedict, W. L.: Protein Therapy in Ophthalmology, Minnesota Med. **11**: 203 (April) 1928. Tobias, G.: Focal Reaction in Eye in Protein Therapy, Klin. Wchnschr. **1**:515 (March 11) 1922.

39. Darier, A.: Pour et contre les injections de lait, Clin. opht. **25**:607, 1921.

40. Shipp, J. M., quoted by Russell (footnote 41).

41. Russel, J. L.: Report of Two Deaths from Third Inoculation with Typhoid Paratyphoid Vaccine; Some Observations on Typhoid Inoculations, Kentucky M. J. **22**:378 (Oct.) 1924.

42. Myers, W. A.: Theory and Practice of Non-Specific Therapy, J. Kansas M. Soc. **20**:30 (Feb.) 1920.

43. Wood, F. C., quoted by Kross: J. M. Research **43**:29 (Jan.) 1922.

44. Lemoine, A. N., in discussion of Allen, T. D.: Typhoid Vaccine in Ophthalmology, Tr. Sect. Ophth., A. M. A., 1925, p. 135.

45. Division of Preventive Medicine: Two Deaths Following Inoculation with B. Typhosus Vaccine, U. S. Nav. M. Bull. **25**:736 (July) 1927.

46. Erdély, G.: Protein Therapy und Appendicitis, Fortschr. d. Therap. **3**: 691 (Oct. 10) 1927.

47. Patton, J. M.: The Pros and Cons of Foreign Protein Injections in Affections of the Eye, J. Iowa M. Soc. **12**:387 (Oct.) 1922.

48. Berneaud: Ueber den Wert der Milchinjektionen bei Augenerkrankungen, München. med. Wchnschr. **1**:1040 (Sept. 5) 1919; Milchinjektionen bei Augen-Leiden, Berl. klin. Wchnschr. **1**:887 (Sept. 15) 1919.

49. Howard, H. J.: Non-Specific Protein Therapy in Eye Inflammations with Special Reference to the Use of Typhoid Vaccine, China M. J. **41**:395 (May) 1927.

for neuroretinitis by Boyd [50] and others; for influenzal pneumonia by Wells,[51] Roberts and Cary [52] and by Cowie and Beaven; [53] in the presence of albuminuria by DeCourcy; [54] in gastric and duodenal ulcers by Pribram,[55] Schmidt [56] and E. F. Mueller; [57] in cholecystitis and gout by Schmidt, and in diabetes by Schmidt and by Fischer and Wichmann-Krause.[58] At the clinic Bargen and I have used protein with safety, at times with definite relief, in cases of arthritis associated with ulcerative colitis.

"It becomes clearly evident," then, as Petersen [20] wrote, "that the identical reaction may alter an inflammatory focus in seemingly diametrically opposite ways, restitution without suppuration, as well as acceleration of suppuration with absorption following."

Clinical Classification.—Clinical expression of these so-called focal or "herd" reactions, has been classified by Schmidt and Petersen under three headings: (1) stimulation of inflammatory foci of infectious origin; (2) stimulation of inflammatory foci of noninfectious origin, and (3) stimulation of latent diathetic phenomena.

Stimulation of inflammatory foci of infectious origin may occur at the site of the active primary inflammation, such as the joints in arthritis, the eye in ocular infections, and so forth. It may occur at the site of active subsidiary inflammation, such as was seen in the enteritis that appeared in case 9 and in the exacerbation of dental infection and disease of the gallbladder in case 7. It may occur, also, in latent (inactive) and unsuspected foci. Here would be listed the majority of my cases, and the reactions would be illustrated by the adenitis in cases 3 and 4, by the appendicitis in cases 8 and 10, by the cholecystitis in case 6, by the respiratory infections in cases 1 and 2 and by the ocular infections in case 11. In certain fields, particularly in genito-

50. Boyd, William: Some Uses of Nonspecific Protein Therapy, J. Lab. & Clin. Med. **5**:88 (Oct.) 1919.

51. Wells, C. W.: Intravenous Injections of Foreign Protein in Influenzal Pneumonia, J. A. M. A. **72**:1813 (June 21) 1919.

52. Roberts, Dudley; and Cary, E. G.: Bacterial Protein Injections in Influenzal Pneumonia, J. A. M. A. **72**:922 (March 29) 1919.

53. Cowie, D. M., and Beaven, P. W.: Nonspecific Protein Therapy in Influenzal Pneumonia, J. A. M. A. **72**:1117 (April 19) 1919.

54. DeCourcy, Carroll: Observations in Non-Specific Protein Therapy, Ohio State M. J. **20**:86 (Feb.) 1924.

55. Pribram, B. O.: Proteintherapie und chirurgische Therapie des Magen- und Duodenalgeschwürs, Deutsche med. Wchnschr. **1**:141 (Jan. 23) 1925.

56. Schmidt, R.: Protein Therapy in Digestive Disturbances, Med. Klin. **20**: 1678, 1924; abstr., J. A. M. A. **84**:238 (Jan. 17) 1925.

57. Mueller, E. F.: Non-Specific Protein Therapy; Its Action and Its Application, Wisconsin M. J. **26**:287 (June) 1927.

58. Fischer, Anton; and Wichmann-Krause: Stoffwechselstudien über unspezifische Therapie, Ztschr. f. klin. Med. **106**:717, 1927.

urinary work, such focal reactions have been deliberately invoked as provocative tests of distinct diagnostic and therapeutic merit.[59]

Under stimulation of inflammatory foci of noninfectious origin have been grouped ocular and renal lesions, such as nephritic retinitis and pain at the site of recent fracture.

Under stimulation of latent diathetic phenomena have been grouped attacks of asthma, epilepsy, gout, tabetic crises and delirium tremens among patients who are victims of chronic alcoholism.

Mechanism of Focal Reactions.—Further experience with and study of unusual reactions will be necessary to determine the correctness of the clinical classification just given. It seems difficult to place in this clinical grouping such reactions as acute vascular thrombosis, anuria and glaucoma occurring in apparently normal eyes. The production of massive thrombi probably requires preexisting vascular disease. Brown also has concluded that it probably requires arteriosclerotic vascular disease, as it has never occurred in uncomplicated cases of thromboangiitis obliterans in which protein therapy was used. However, Hektoen and Irons [60] reported a severe reaction and pulmonary embolism occurring immediately after a subcutaneous injection of polyvalent stock vaccine for thrombophlebitis.

Allergy or anaphylaxis as the cause of focal reactions has been suggested, among others, by Kolmer [61] and by Gottschalk.[62] Apart from the massive thrombi seen in these cases of vascular disease, it must be remembered that small thrombi are part of the pathologic manifestations of anaphylactic and anaphylactoid events. It has been suggested that all of these untoward reactions have as their common pathologic basis small thrombi which, in turn, are due to a common immunologic alteration, such as allergy or anaphylaxis. Sollmann, however, has expressed the belief that foreign protein therapy does not elicit an immune reaction but a pharmacologic reaction.

Details concerning the sudden "anaphylactic" deaths reported are, in general, meager. Although some have expressed the belief that

59. Holloway, J. K., and Von Lackum, W. H.: Chronic Prostatitis with Special Reference to Its Focal Aspects, M. J. & Rec. **122**:64 (July 15) 1925. Müller, Rudolf: Die Behandlung des venerischen Bubo mit Milchinjektion, Wien. klin. Wchnschr. **32**:780 (July 10) 1919. Neverman, Hans: Eine intrakutane Provokations-Methode bei der weiblichen Gonorrhöe, München. med. Wchnschr. **1**:141 (Feb. 4) 1931.

60. Hektoen, Ludvig; and Irons, E. E.: Vaccine Therapy, J. A. M. A. **92**:864 (March 16) 1929.

61. Kolmer, J. A.: A Practical Text-Book of Infections, Immunity and Biologic Therapy with Special Reference to Immunologic Technic, ed. 3, Philadelphia, W. B. Saunders Company, 1924.

62. Gottschalk, Alfred: Umstimmung des Zellstoffwechsels als Grundlage pathologischer Reaktionen, Klin. Wchnschr. **1**:109 (Jan. 15) 1923.

anaphylaxis does occur after protein therapy (Nakazawa,[63] Valléry-Radot,[64] Teague and McWilliams,[65] van Randenborgh,[66] Kross[67]), others have expressed their belief that true anaphylaxis does not occur, in that eosinophilia or bronchial spasm are not evident (Miller and Lusk,[3] Cowie and Calhoun,[26] Greenthal and Brown[68]).

Careful review of the past medical experiences of patients seen in the clinic did not reveal a detectable, significant, common denominator. There was no history of hypersensitivity to protein, known allergy or urticaria. Only two patients had had typhoid fever and these not within recent years.

A brief study of the mechanism of production of the focal reactions, as summarized by Petersen, may help to explain some of the accidents of protein therapy. The injection initiates a sharp clinical departure from the physiologic equilibrium. The most important feature of the reaction is its dual nature, expressed in two phases. The symptoms of the first (negative) phase are increased inflammation, pain, irritability, redness and swelling at the sites of active or latent inflammation. Four main factors are believed to produce these symptoms: (1) An increase in capillary permeability and increased exudation of tissue fluids occur; redness and swelling result; (2) increased tissue pressure is produced, and there is lowering of the threshold for nervous stimuli; increased pain and tenderness occur; (3) there follow increased digestion of inflammatory tissue at foci of circumscribed infection and inflammation; localization of focal reactions is aided by the fact that cells previously involved in an inflammatory reaction respond more readily to all stimuli, and (4) if the resultant necrosis of tissue is great,

63. Nakazawa, F.: Influence of Parenterally Administered Protein Substance upon Respiration of Tissue Cell, Tohoku J. Exper. Med. **5**:546 (March) 1925.

64. Valléry-Radot, Pasteur: Diagnostic des affections anaphylactiques par les cuti-réactions; méthodes de traitement des affections anaphylactiques, en particulier par la peptonothérapie, Rev. méd. franç. **8**:39 (Jan.) 1927.

65. Teague, Oscar; and McWilliams, Helen I.: The Bacteriolytic Power of Normal and Immune Rabbit Serum for Typhoid Bacilli and the Influence of the Intravenous Injection of Vaccine upon the Same, J. Immunol. **2**:167 (Feb.) 1917; Experiments with a Possible Bearing upon Treatment of Typhoid Fever with Typhoid Vaccine Administered Intravenously, ibid. **2**:185 (Feb.) 1917; The Bacteriolytic Power of Normal Human Sera and Typhoid Patients' Sera for Typhoid Bacilli and an Inquiry into the Theoretical Basis for the Treatment of Typhoid Fever with Vaccine Administered Intravenously, ibid. **2**:193 (Feb.) 1917.

66. van Randenborgh, A.: Anaphylaktische Erscheinungen bei Proteinkörper-Therapie, Zentralbl. f. Gynäk. **44**:1128 (Sept. 25) 1920; Anaphylactic Manifestations in Proteotherapy, Zentralbl. f. Gynäk. **44**:1128, 1920; abstr., J. A. M. A. **76**:418 (Feb. 5) 1921.

67. Kross, Isidor: An Experimental Study on the Effects of Protein Injections upon Infections, J. M. Research **43**:29 (Jan.) 1922.

68. Greenthal, R. M., and Brown, G. M.: Studies on the Nature of Nonspecific Protein in Disease Processes, Arch. Int. Med. **30**:99 (July) 1922.

there is marked systemic autointoxication from absorption of digested material.

The departure from the physiologic equilibrium somehow stimulates a reverse phenomenon which not only restores but overcorrects the inflammatory status, and partial or complete healing is obtained. The various theories regarding the exact mechanism of this stimulation cannot be given. It was Weigert [69] who first stated that local necrosis at some part of a living organism will, as a rule, result in heightened functional activity. But Arndt [70] provided the corollary that although weak stimuli accelerate vital activities, strong stimuli inhibit them, and maximal stimuli suppress or utterly destroy them. Hence, if the lytic phenomena of the first phase are excessive, necrosis may get beyond control, without the healing phenomenon of the second phase to compensate for it. In one case the negative phase may be scarcely obvious and the positive phase may rule the picture. Indeed, Cowie has expressed the belief that, in arthritis at least, as far as a reduction of antibody content is concerned, a negative phase is not induced. In another case the opposite may occur, and a positive phase does not seem to follow the negative phase.[71] Then a chronic progression of the inflammatory state may be the deleterious result of protein therapy. Then ulceration, even perforation of certain structures, such as the appendix or cornea, may occur. Thus, as Maliwa [10] has expressed it, the art of protein therapy is not to exceed the desirable limits of this inflammatory process. According to Garrison,[9] "The crux of the matter is the specific reaction of the individual patient to the threshold dose."

I am still not certain whether these explanations are fully adequate to account for the production of all unusual reactions. Although a change in capillary permeability is among the features noted as part of the normal reaction, such a change is probably usually relatively slight. This change would have to be unusually profound to produce vascular thrombosis, glaucoma and anuria in the presence of normal vascular, ocular and renal function. One must either assume that function or histologic structure was not entirely normal, or that these unusual alterations of function were induced by unusual alterations in permeability. Perhaps, then, a fourth clinical grouping should be recognized, namely, reactions dependent on some marked vasomotor or intravascular chemical change, or on profound disturbances in capillary permeability or colloidal dispersion. This group would include chiefly the vascular accidents, and certainly would provide the most acute, and most of the fatal, reactions.

69. Weigert, quoted by Garrison (footnote 9).

70. Arndt, quoted by Garrison (footnote 9).

71. Laufer, Otto: Ueber die Möglichkeit von Schädigungen durch Protein-Körperinjektionen, Med. Klin. **2**:1366 (Sept. 3) 1926.

Studies of blood chemistry indicate that no definite burden is put on the kidneys by the reaction, even though many minor variations in renal function are produced. It was reported by Fremont-Smith [72] that in the first phase a degree of antidiuresis is produced that may at times approach anuria. In the second phase antidiuresis may continue, or diuresis may begin and may continue the subsequent day. My studies on the excretion of phenolsulphonphthalein at different stages in the reaction disclosed that the excretion of dye may be reduced as much as 50 per cent during the second phase, with a subsequent prompt return to normal. These alterations in renal function may have a vascular basis.

Petersen and Miller expressed the belief that vasodilatation of renal vessels occurs in the first phase; vasoconstriction, in the second. Mendelsen's [73] expressed opinion was that the renal vessels are constricted, but at what stage, he did not mention. The general vascular changes include reduction of peripheral blood pressure in the first and second phases; at times, considerable reduction in the latter phase. Capillary permeability is thought to be increased in the first phase and decreased in the second phase. The condition of the cutaneous capillaries is not definite. Fremont-Smith and his co-workers reported marked stasis of capillary blood in the first stage and rapid flow in the second. Brown [74] has noted variability, depending on whether the patient had normally warm or normally cold hands. In general, there was a slow flow, or stasis, in the prodromal (latent and chill) phase, with increased flow in the subsequent phases. It was the slowing of the peripheral flow of blood in the first stage to which Allen and Smithwick attributed thrombus formation.

Although the fatal anuria in one of my cases was in association with iliac thrombi, and in another with a diffuse renal lesion, in the third case there was neither nephritis nor thrombus. Antidiuresis is concurrent with fever, but in this case there was no prolonged fever. It is but speculation to suggest that the anuria was the result of a disturbance of renal vascular (vasomotor) physiologic processes which was not followed by reestablishment of the normal condition. If it is true that the vasomotor status in the viscera is opposite to that in the periphery in the first stage, and that both undergo, in the second stage, complete reversal to the opposite status, one might suggest either that an extensive vasomotor or intravascular chemical change, resulting in thrombosis, or that a vasomotor imbalance set in a state of irretrievable

72. Fremont-Smith, Frank; Morrison, L. R., and Makepeace, A. W.: Capillary Blood Flow in Man During Fever, J. Clin. Investigation **7**:489 (Aug.) 1929.

73. Mendelsen, Walter: On the Renal Circulation During Fever, Am. J. M. Sc. **86**:380 (Oct.) 1883.

74. Brown, G. E.: Personal communication to the author.

decompensation is induced. These possibilities are particularly tenable in cases of vascular disease, for instance, of arteriosclerosis.

Relation of Dose of Vaccine to Reactions.—Dosage seems to be of variable significance in the production of undesirable reactions. Untoward reactions have occurred after very small doses administered intramuscularly as well as intravenously. In my series the reactions all followed small doses. Contrarily, many physicians have used purposely, and others by mistake, very high doses with impunity. Cowie and Calhoun, Howard and others repeatedly used intravenously, without accident, doses as high as from 1,500,000,000 to 2,500,000,000 typhoid organisms. Although the dose in the fatal case reported by Irons [17] was high (5,000,000,000 organisms), he and Jobling [75] have stated that similarly high doses of various organisms have been given without apparent harm. The size of the dose only, therefore, cannot be blamed for unusual reactions.[76]

Regarding high doses, let me note here an unusual reaction that occurred elsewhere subsequent to an injection of 2,000,000,000 typhoid and paratyphoid bacilli by mistake. A patient with thrombo-angiitis obliterans was seen at the clinic in 1928. After fair relief had followed an initial injection of 125,000,000 bacilli, he was advised to have similar treatment continued at home. Through a misunderstanding on the patient's part, the physician at the patient's home was induced to give two injections, each of which contained enormous doses. The second dose was later estimated to have been of at least 2,000,000,000 organisms. The reaction was intense. A temperature of from 104 to 106 F. lasted for three weeks. Gangrene of the right foot promptly occurred, and amputation was necessitated a few weeks later.

Although large doses may be tolerated, small doses if accompanied by an adequate reaction, seem preferable, since as beneficial results seem to follow the use of the small doses as the large doses. Regarding frequency of doses, Howard [49] reported giving, without harm, fifty-seven injections intravenously to a patient over a period of eleven months.

Contraindications.—There are certain contraindications to treatment by foreign protein. These are as follows: (1) marked arteriosclerosis, peripheral or renal; (2) uncompensated cardiac (Cowie), renal (Miller) or vasomotor (Cecil) disease; (3) chronic infections of long duration, with dissemination to several sites; (4) states of exhaustion following prolonged illness (Miller); (5) pulmonary tuberculosis, active or quiescent; (6) conditions in which hemorrhage may occur, such as

75. Jobling, J. W.: The Influence of Nonspecific Substances on Injections, Arch. Int. Med. **19**:1042 (June) 1917.

76. The American and French billion equals one thousand million, and the English and German billion equals a million million.

hemophilia, in patients subject to frequent bleeding, such as epistaxis, and in the ulcerating stage (third week) of typhoid fever (Snyder,[77] Jobling); (7) the period within fourteen days after an operation, for hemorrhage has occurred occasionally when vaccine was given within ten days after tonsillectomy or minor pelvic operations; (8) marked hypertension (Miller); (9) chronic alcoholism, for fear of delirium tremens (Scully, Müller); (10) states of grave nervous instability (Scully), quiescent or active, such as hyperthyroidism (Miller), epilepsy and certain psychopathic conditions other than dementia paralytica; (11) definite protein hypersensitivity, suggested by a history of serum-sickness, urticaria or angioneurotic edema (Betz[78]); (12) pregnancy, for fear of inducing hemorrhage and uterine muscular contractions (Snyder, Betz, Ichikawa); (13) rheumatic fever with acute or decompensated carditis (compensated cardiac disease has not been considered a contraindication [Miller]), and (14) diabetic acidosis (Woods[79]); the treatment has been safely used at the clinic in controlled diabetes coexistent with arthritis.

Certain investigators do not consider endocarditis a contraindication. Miller has tried protein therapy in cases of septic endocarditis, and Gross[80] and Cecil have employed it safely in cases of rheumatic fever with endocarditis. Others feel that it can be used with safety, if with caution, in hypertension, pregnancy (Campbell), disseminated infection, typhoid fever (Fairley, Miller), hypersensitivity, menstruation (Campbell) and severe anemia (Miller). Infancy and age (Laurie) have not been considered contraindications, provided that with age the vascular system particularly seems materially sound.

Regarding the safety of protein therapy in pulmonary tuberculosis there are different opinions. Schmidt and Kraus[81] and Campbell reported pulmonary focal reactions in tuberculous patients, and Matthes[82] in tuberculous animals, after injections of protein. In the review of Hektoen and Irons are cited five deaths from activation of latent pulmonary tuberculosis by the use of stock mixed vaccines. On

77. Snyder, R. G.: A Clinical Report of Nonspecific Protein Therapy in the Treatment of Arthritis, Arch. Int. Med. **22**:224 (Aug.) 1918.

78. Betz, Isidor: Treatment of Arthritis by Nonspecific Therapy, M. Rec. **99**: 920 (May 28) 1921.

79. Woods, A. C.: Protein Therapy, Specific and Nonspecific in Ophthalmology, Ohio State M. J. **23**:740 (Sept.) 1927.

80. Gross, J. G.: Foreign Protein Injections in the Treatment of Acute Infections, Journal-Lancet **37**:764 (Nov. 15) 1917.

81. Schmidt, R., and Kraus, Otto: Ueber Proteinkörpertherapie bei Tuberkulose, Med. Klin. **1**:503, 1919.

82. Matthes, quoted by Petersen, W. F.: Factors in Resistance to Tuberculosis, Arch. Int. Med. **21**:14 (Jan.) 1918.

the other hand, in experiments on animals Baldwin and L'Esperance [83] believed increased fibrosis and resistance were provided, whereas Brown, Heise, Petroff and Wilson [84] noted focal activation in only 2 of 124 tuberculous patients given antityphoid immunization. Similar safety was reported by Clovis and Mills.

Unusual reactions have been noted much more frequently on the arthritic and vascular services than elsewhere at this clinic. In the section on dermatology and syphilology much larger doses are given than on the arthritic service, yet untoward reactions are rare. In certain intractable cases of neurosyphilis, progressive doses reach the amount of several billion organisms given intravenously. In a very few cases, petechiae, jaundice or reactivation of malaria have been noted.[85] This would seem to emphasize what was stated previously, that the reaction does not depend only on the material or dose used, but on the type and extent of the patient's illness. In severe chronic infectious arthritis it is recognized that the pleura, pericardium, spleen and lymph nodes often endure the disseminated infection or inflammation. Such dissemination may explain the preponderance of reactions in this group of patients. In these cases, especially when there is a history of recent pleurisy, pericarditis, myocarditis or abdominal infection, special care may be necessary. There must be some regenerative power left in affected tissues to obtain good results. Although exhaustion and disseminated inflammation may mitigate against the best results, one may not always be justified in withholding the possible benefits of such treatment in those cases in which relief is most desired and difficult to obtain, and when previous treatment has failed (Cecil).

Prevention of Untoward Reactions.—Prevention of untoward reactions may be possible. It is dependent on (1) familiarity with the substance used; (2) moderate dosage; (3) careful selection of patients; (4) care of the patient during the reaction, and (5) continuation of the treatment only if the reactions are well borne and satisfactory relief is obtained.

Most important is the selection of patients with due regard to the contraindications noted. Inquiry into the history may suggest the possibility of the presence of quiescent infection from past illnesses. In the presence of marked exhaustion, disseminated infection with little evidence of resistance on the patient's part, or marked arteriosclerosis,

83. Baldwin, H., and L'Esperance, E. S.: The Influence of Typhoid Vaccine on Tuberculous Guinea-Pigs, J. Immunol. **2**:283 (April) 1917.

84. Brown, Lawrason; Heise, F. H.; Petroff, S. A., and Wilson, G. E.: A Study of the Effects of Typhoid Fever and Antityphoid Immunization on Pulmonary Tuberculosis, Am. Rev. Tuberc. **2**:717 (Feb.) 1919.

85. O'Leary, P. A., and Goeckerman, W. H.: Personal communication to the author.

protein therapy by a more conservative method than the intravenous injection of vaccine should be the method of choice. The vascular and vasomotor alterations induced by a marked reaction may be poorly borne. The proteins that entail milder reactions and the more conservative (intramuscular) method of administration may be permitted.

Although hospitalization during treatment is advisable for patients who are seriously ill, or if it is suspected that an untoward reaction or focal stimulation might occur at the clinic, treatment is administered to large numbers of selected persons who remain as outpatients. Patients should be advised what constitutes the symptoms of a normal reaction, so that unusual phenomena may not be ignored.

To avoid gastro-intestinal symptoms if possible, it is better that no food should be taken from two to four hours prior to an injection. However, many tolerate injections without such restriction of food.

The patient should go to bed within a half hour after the injection, and remain there during the reaction. To lessen the discomforts of the reaction, especially the period of chill, hot water bottles and mild analgesics should be supplied. It has been noted that the local application of heat prevents stasis of the blood in peripheral capillaries, which occurs in the first stage, or it abolishes stasis once it has occurred. Fluids should be given during the treatment, especially if reactions are associated with definite loss of weight.

The reaction can be shortened and discomfort lessened, according to Scully,[24] Myers [42] and Perkins and White [86] by preceding the injection with a small dose of codeine or morphine given hypodermically, and by giving from 5 to 10 minims (0.31 to 0.61 Gm.) of epinephrine chloride (1 : 1,000 solution) subcutaneously after the chill. Heat may suffice instead of epinephrine. It may be argued, however, that minimizing the reactions thus may lessen the chances for ultimate benefit. Duke [87] has expressed the belief that unusual reactions may be prevented by mixing very small doses of epinephrine and ephedrine with the vaccine.

Early Recognition.—In eight of my fourteen cases in which unusual reactions occurred, the reaction appeared after the initial dose. In the experience of Zimmer and Buschmann,[88] focal reactions usually occur after the first injection and, if they recur, may be of minimal severity, and finally may cease to occur. The approach of unusual reactions frequently can be recognized by attention to unusual complaints on the part of the patient. Pain in the thorax or precordium may signalize

86. Perkins, R. J., and White, G. B.: Rheumatoid Arthritis Treated with Intravenous Bacillus Coli Vaccine, Brit. M. J. **1**:411 (March 10) 1923.

87. Duke, W.: Personal communication to L. G. Rowntree.

88. Zimmer, Arnold; and Buschmann, Paul: Aus der chirurgischen Universitäts Klinik, Ztschr. f. ärztl. Fortbild. **22**:513, 1925.

pleurisy or pericarditis. Unusual degrees of nausea and anorexia or the appearance of diarrhea or of localized abdominal tenderness may mark the onset of enteritis or focal activation in the appendix, gallbladder or pelvis. A sharp, continued pain in the region of a large blood vessel during the period of chill, or at the end of it, may denote the formation of thrombi. If the patient's fever continues longer than from eighteen to twenty-four hours after the injections, reexamination of the thorax and abdomen is indicated. The leukocytosis (from 15,000 to 40,000 leukocytes per cubic millimeter of blood) induced by the reaction usually disappears after from twenty-four to forty-eight hours. If it remains high, an explanation should be sought. An adequate output of urine the day of the injection and for two days subsequent to it, should be inquired for and insured.

Treatment of the Reaction.—The treatment in cases of unusual reaction does not differ in general from the treatment of such phenomena when they are not induced by protein therapy. When frank instability of vascular physiologic processes occurs, it should be treated on the basis of the knowledge of the vasomotor alterations induced, particularly in the first or negative phase of the reaction. If vascular accidents occur, such as thrombi or vasomotor depression, the patient should be kept in bed, and warm applications should be used. If possible, thrombi in peripheral vessels should, of course, receive prompt surgical care. Gow,[89] stressing the possible anaphylactic nature of some of the reactions, relieved dyspnea and cyanosis by injections of epinephrine (0.75 cc. of a 1:1,000 solution) or one-hundredth grain (0.6 mg.) of atropine sulphate. In this connection the experimental work of Hanzlik and Karsner[90] on the prevention or amelioration of anaphylactoid phenomena from intravenous therapy by the use of atropine and epinephrine is of interest. If renal insufficiency and oliguria occur, the intravenous administration of diuretics is indicated.

The activation of latent inflammatory foci, if kept within control, should not be looked on as an untoward reaction. Indeed, it should be regarded as a fortunate demonstration of unsuspected infection. Thus the procedure constitutes a legitimate provocative test. Theoretically, the possible harmful phenomena of the first phase are safely reversed by the second phase. It may not always be safe to rely on this, however. For example, if acute appendicitis is discovered, the usual surgical indications prevail.

89. Gow, A. E.: Intravenous Protein Therapy, Brit. M. J. **1**:284 (Feb. 28) 1920.

90. Hanzlik, P. J., and Karsner, H. T.: Anaphylactoid Phenomena from the Intravenous Administration of Various Colloids, Arsenicals and Other Agents: A Comparison of the Prophylactic Effects of Atropine and Epinephrine in Anaphylactic Shock and Anaphylactoid Phenomena from Various Colloids and Arsphenamine, J. Pharmacol. & Exper. Therap. **14**:379 and 425 (Jan.) 1920.

SUMMARY AND CONCLUSIONS

The reactions to injections of foreign protein (typhoid vaccine intravenously) have been studied in a group of about 2,500 patients, representing an aggregate of about 10,000 injections. Of these 2,500 patients approximately 1,500 were on the arthritic service and 1,000 on the vascular and other services.

The reactions have, in general, been well borne, and the beneficial results from protein therapy justify, in certain diseases at least, its continued use and further development.

Unusual reactions to such treatment have been rare at the clinic. They have occurred in this series in fourteen cases in all, an incidence of about 0.5 per cent of the patients. They occurred seventeen times in the treatment of twelve patients with arthritis (including one patient with rheumatic fever) and three times in the treatment of two patients with occlusive vascular disease. Thus, of 10,000 injections only twenty were followed by an unusual reaction, an incidence of 0.2 per cent of the injections.

Unusual reactions subsequent to the injections were as follows: acute and subacute appendicitis, cholecystitis, enteritis, pleurisy, pericarditis, iritis, glaucoma, adenitis, extensive vascular thrombosis and renal insufficiency. In arteriosclerotic vascular disease, acute thrombosis is the occasional possibility. In arthritis and other diseases, the reactions are variable. It is believed that these reactions are not incidental complications, but are related to protein therapy in the presence of an underlying disease.

Death occurred in three instances, a mortality rate of 0.12 per cent. This constitutes a very small risk, but a risk that must be recognized and that can possibly be avoided by the most careful selection of patients.

Unsuspected latent or quiescent focal inflammation and infection may be stimulated. Except in certain conditions, of which pulmonary tuberculosis is one, the known presence of latent or quiescent foci should not act as a contraindication to such treatment. Indeed, part of the value of such treatment lies in the possible demonstration of suspected or unsuspected foci otherwise undemonstrable at the time. Such reactions, if their significance is appreciated, may be advantageous rather than detrimental.

The mechanism of the usual and unusual reactions can be understood, in part at least, from study of the various reported components of the reactions.

Unusual reactions have been considered previously as being of three types, for they resulted mainly in one of the following: (1) stimulation of inflammatory foci of infectious origin, (2) stimulation of inflammatory foci of noninfectious origin or (3) stimulation of latent diathetic

phenomena. There may be a fourth type of unusual reaction, one dependent on some marked vasomotor or intravascular chemical change which may produce acute thrombosis or vasomotor imbalance. Advanced sclerosis of peripheral coronary or renal arteries must be stressed as a possible contraindication in addition to those mentioned by others. Particularly must this be taken into consideration in the treatment for occlusive arteriosclerotic vascular disease of the extremities.

The prevention, recognition and treatment of unusual reactions is considered.

The assembly here of the unusual reactions experienced at the clinic and elsewhere, it is hoped, will not give them an undue emphasis, to the detriment of a useful and essentially safe form of treatment. The recognition that such reactions may occur has led me to a more careful selection of patients and a more judicious use of protein therapy.

THE QUANTITATIVE DETERMINATION OF THE POTENCY OF LIVER EXTRACT *

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In a previous communication [1] it was demonstrated that liver extract had a marked influence in ameliorating the signs and symptoms of pernicious anemia of the fowl. The present paper deals with the same subject on a quantitative basis.

As already discussed,[1] a disease complex, analogous in all important respects to pernicious anemia in human beings, can be separated from the pernicious anemia-leukemia syndrome of Ellermann [2] as it occurs in fowls. Moreover, it can no longer be doubted that, in fowls, the leukanemic or aleukemic leukotic cases, that is, cases with symptoms and signs of pernicious anemia but with myelocytic proliferations in various organs, especially in the liver, belong essentially to the pernicious anemia group and not to the leukotic one. Pernicious anemia, therefore, as it occurs in the fowl, is quite a distinct and separate disease in every respect, especially as regards etiology, from leukemia or leukosis. There are no borderline or transitional cases, such as leukanemias might be considered, bridging the gap between the one state and the other. The present investigation makes use of cases of pernicious anemia so defined as the basis of the experimental work.

The fowls employed were white leghorns of various ages, the subjects of a spontaneous attack of the disease. The condition in the different cases had progressed to varying degrees of severity.

While under observation, they were confined singly in cages, inside a house. They were fed, ad libitum, on a bran and sharp mash and mixed grains, and had free access to water and oyster shell grit. As they were still on the same ration as before but no longer on free range, their conditions were not so liberal as those under which they had contracted the disease.

The various medicinal agents were administered twice daily, at 9:30 a. m. and again at 2 p. m. This daily twofold dosing has to be borne in mind in reading the accompanying charts, which indicate only the points where a certain treatment or change of treatment was com-

* Submitted for publication, April 8, 1931.

* From the Rowett Research Institute.

1. McGowan: Edinburgh M. J. **37**:330, 1930.

2. Ellermann: The Leucosis of Fowls and Leukaemia Problems, Gyldendal, 1921.

menced. As a rule the fowls received any one dose for two days before a change was made to the next higher one.

The observations recorded were made for the purpose of arriving at the smallest dose of liver extract which would produce a noticeable and significant effect on the blood curves. They deal, thus, with the effect of administration of liver extract in conjunction with iron and ammonium citrate by mouth; of liver extract intraperitoneally with iron and ammonium citrate orally, and finally of liver extract intraperitoneally without iron. The description follows the same sequence. The amount of iron and ammonium citrate administered was constant throughout the series and consisted of 0.5 Gm. given twice daily.

The liver extract employed was no. 343, in three batches.[3] When given by mouth it was dissolved in water and administered by means of a rubber teat pipet; when intraperitoneally, it was suspended in ether—the ether being allowed to evaporate slowly—dissolved in sterile saline solution and injected.

In the description of the results obtained in this investigation, much of the important information gleaned has been incorporated in the charts. A short explanation, therefore, of each chart with a discussion of points special to each case will be all that is required by way of letterpress. As it is not germaine to the present issue, no cognizance will be taken, in the description of bone marrow changes, of the finer details, such as the alteration from a hemocytoblastic-amitotic to an erythroblastic-mitotic basis in the formation of the erythrocytes.[1] The description will center round what is more important in the present instance, the occurrence of sclerosis of the bones, the disappearance of the marrow tissue, and the question as to whether the marrow, histologically, is in a normal, "hyperplastic" [4] or aplastic condition.

DESCRIPTION OF CASES

Hen 344 (chart 1).—On the third day after the commencement of the administration twice daily of one-fourth phial [5] of liver extract by mouth, there was a slight rise of both the hemoglobin and red blood cell curves, which persisted for a short time. The subsequent administration of one-fourth phial, intraperitoneally, however, produced no effect. It demonstrated, however, that the extract could with care be given intraperitoneally, without injurious local effect.

The marrow in this case was in a very aplastic condition—indeed, in the tibia it had entirely disappeared, leaving large fatty oily cavities filled with a clear fluid. There was considerable sclerosis of the bones. In all probability the

3. Supplied by Eli Lilly & Company, Indianapolis.

4. Hyperplasia of the pernicious anemia type.

5. To avoid the repetition of this cumbersome phrase, future references will merely give the fraction and the phial and leave the rest to be understood. The word phial refers to the receptacle in which each dose is separately contained for human use.

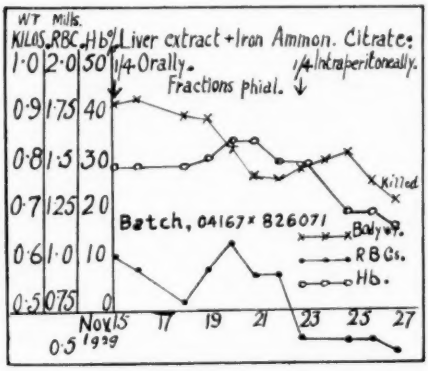

Chart 1.—Results in hen 344.

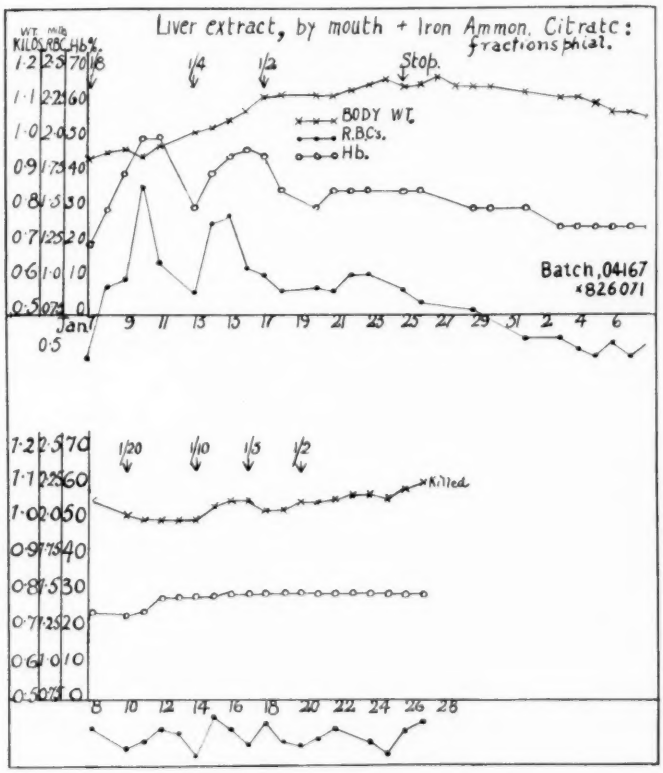

Chart 2.—Results in hen 350.

degeneration of the marrow was responsible for the small and fleeting reaction obtained and for the ineffectiveness of the subsequent intraperitoneal injections.

HEN 350 (chart 2).—This fowl showed an almost immediate and very considerable reaction to the administration of one-eighth phial orally. Though this dose was continued, there was a marked fall in the curves in a few days. This was followed by a rise on increasing the dose to one-fourth phial. In its turn this was followed by a fall which persisted in spite of the dose being raised to one-half phial. On January 25, the liver extract and the iron were stopped and the hemoglobin and red blood cell curves continued to fall. On February 10, treatment was recommenced, this time with very small doses of the liver extract. The most that can be said with regard to its effect on this occasion is that there was a slight rise in the hemoglobin curve while the red blood cell curve became more unstable and irregular.

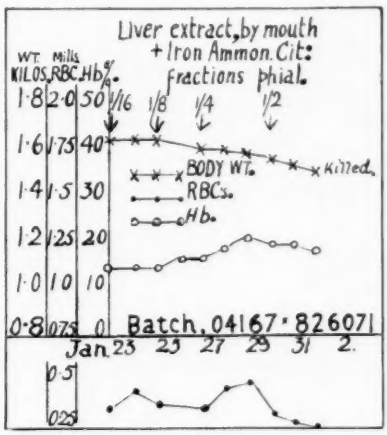

Chart 3.—Results in hen 353.

On being killed on February 26, the fowl was found to be suffering from marked ascites. This in all likelihood was responsible for some of the increased weight observed. There was a fair amount of sclerosis of the bones, with a reduction in the quantity of marrow. The marrow itself was hyperplastic in type.

HEN 353 (chart 3).—In this case there was a very slight and doubtful response in the hemoglobin and red blood cell curves, which began with the administration of one-eighth phial. The bones were considerably sclerosed and the marrow was in the same aplastic and degenerated condition as in hen 344.

HEN 358 (chart 4).—In this case the first definite rise in the red blood cell and hemoglobin curves occurred on the administration of one-fourth phial by mouth. This was followed by a more definite rise when one-half phial was given. Later both curves sank considerably, even though the dose had been raised to 1 phial.

The hen died on March 24. It was greatly emaciated, and considerable ascites was present. There was quite definite sclerosis of the bones with reduction of the quantity of marrow. The marrow itself was in a hyperplastic condition.

Hen 361 (chart 5).—In this case the red blood cell and hemoglobin curve commenced to rise on the administration of one-thirty-second phial. This upward tendency increased and reached its height on March 5 during the administration of one-half phial. Shortly after this, on March 9, the curves began to fall and continued to do so although the dose administered was now 1 phial. The dosing was stopped on March 15, when the fall became accelerated.

The fowl was killed on April 4. There was marked old sclerosis of the bones with a great reduction in the quantity of the marrow. The marrow itself was

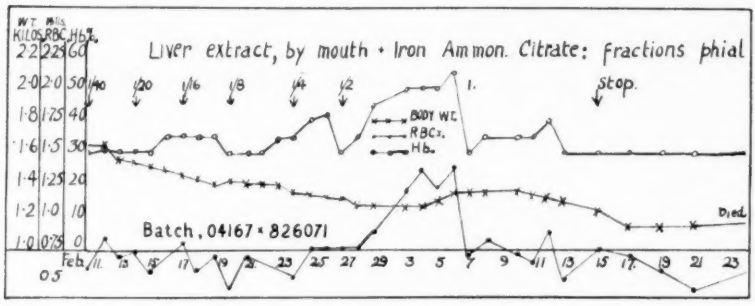

Chart 4.—Results in hen 358.

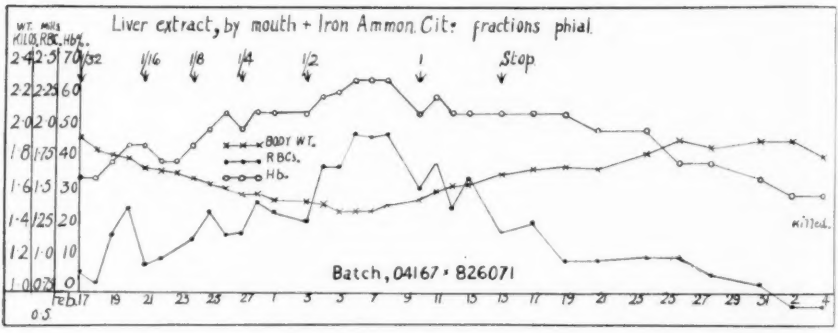

Chart 5.—Results in hen 361.

of a hyperplastic type. This fowl was fat and muscular and showed no edema or ascites.

Hen 362 (chart 6).—Here there was a small ephemeral rise in both curves subsequent to the administration of one-half phial by mouth. This was followed by a fall which persisted even though the dosage was increased to 1 phial.

The fowl was killed on April 2. It was muscular and fat and without ascites. There was very great sclerosis of the bones with reduction of the quantity of marrow. The marrow itself was in a state of great aplasia, consisting as it did for the most part of fat cells only. This would account for the lack of response in the blood curve.

HEN 363 (chart 7).—Here there was no response, and the hen died in a few days. In this case the bones were so sclerosed that there was practically no marrow left. What was present was in a condition of advanced aplasia.

HEN 369 (chart 8).—In this case, too, there was no response to the administration of liver extract.

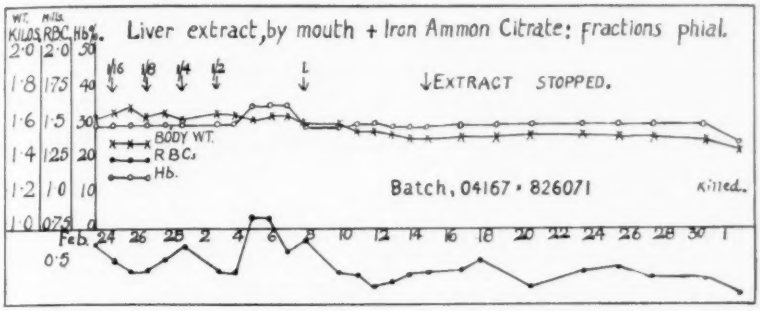

Chart 6.—Results in hen 362.

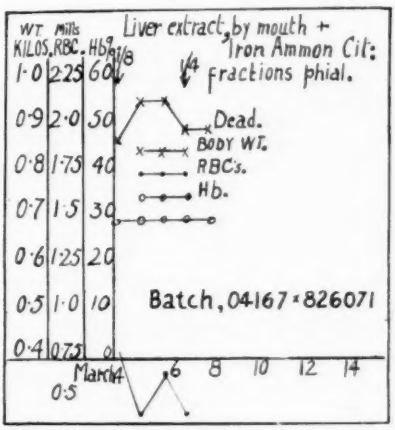

Chart 7.—Results in hen 363.

The fowl was killed on April 7. The bones were in a condition of advanced sclerosis, and the marrow had practically disappeared. Any that was left was in a condition of advanced aplasia.

HEN 371 (chart 9).—Here the first observation on the blood was made on May 31. An interval elapsed before the next one was made, and the administration of liver extract was commenced on June 22. It is possible that there was a slight response to the administration of one-fourth phial on June 23 and 24. Thereafter there was no reaction in spite of increased dosage and the employment of another batch of the extract.

The fowl was killed on July 8. There was considerable sclerosis of the bones and great reduction of the marrow, which showed marked aplasia.

Hen 377 (chart 10).—This fowl was kept under observation for a considerable period and subjected to varied treatments. Liver extract, batch 04167 × 826071, in a dosage of one-half phial, would seem to have produced a slight response, which increased considerably when a whole phial was given. A change to batch

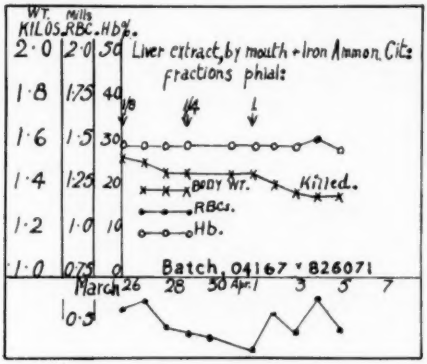

Chart 8.—Results in hen 369.

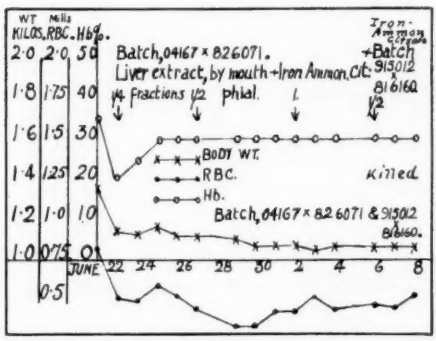

Chart 9.—Results in hen 371.

915012 × 816160 maintained the rise, but a fall, which had already commenced, persisted on changing back to batch 04167 × 826071, even at an increased dosage. A change to ox liver now did not improve matters, but a marked though temporary swing up occurred on June 2 on the intraperitoneal injection of one-eighth phial, and again on June 9 on the injection of one-fourth phial. All treatment was stopped for a while, but on June 30 and again on July 9 the curves were caused to move up by the intraperitoneal injection of one-fortieth and one-fifth phial, no iron being administered on this occasion. The batch employed here was 4 F8 × 842599.

The hen was killed on July 14. There was a considerable accumulation of brown material, possibly inspissated liver extract and necrotic cells, in the peritoneal cavity but no peritonitis. The bones were considerably sclerosed and there was a marked reduction in the quantity of the marrow. This, however, was of a hyperplastic type.

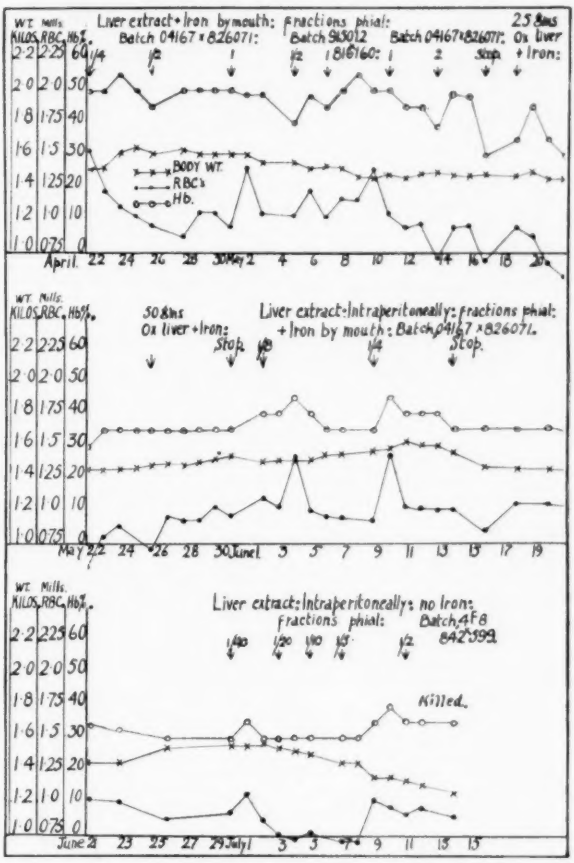

Chart 10.—Results in hen 377.

HEN 378 (chart 11).—This hen received treatment identical with that of hen 377 during the same period of time. It gave, however, a much better response to treatment with liver extract at every stage of the experiment. Attention need only be drawn to a few special points. On April 21, an immediate response was obtained to the administration of one-fourth phial orally. This rise was sustained through varying dosages up to May 16. The administration of ox liver now, at a time when the curves had fallen somewhat, caused a slight rise. This was considerably increased on the intraperitoneal administration of increasing doses of

liver extract beginning with one-eighth phial. However, on June 13, a fall had set in before this treatment was stopped (June 14). The fall continued till July 7, when a rise in both curves commenced again on the intraperitoneal administration of liver extract in increasing doses beginning with one-fortieth phial. No iron was given on the last occasion, and the batch employed was 4 F8 × 842599.

The hen was found dead on July 19. The peritoneal cavity was greatly distended with brown material as in hen 377. There was considerable sclerosis of the

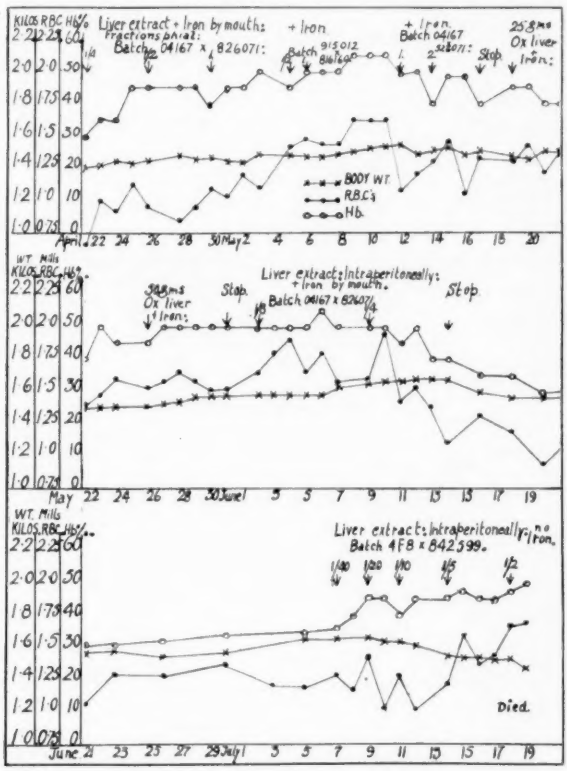

Chart 11.—Results in hen 378.

bones, with reduction of the quantity of marrow. This was of a hyperplastic type.

Hen 379 (chart 12.)—This hen was tested with batches 04167 × 826071 and 915012 × 816160 by mouth with the addition of iron. A response was obtained with one-fourth phial of the first batch; this increased somewhat on increasing doses and was not altered by changing to the second batch.

This fowl was killed on May 7. The bones were found to be so sclerosed that there was hardly any marrow left. What remained was in a very aplastic condition.

HEN 384 (chart 13).—Liver was given in the first instance in this case, followed by liver extract. This was done in an attempt to gage the potency of the one as compared with the other. Little deduction, however, can be drawn

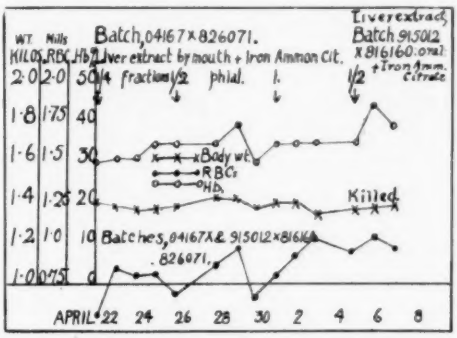

Chart 12.—Results in hen 379.

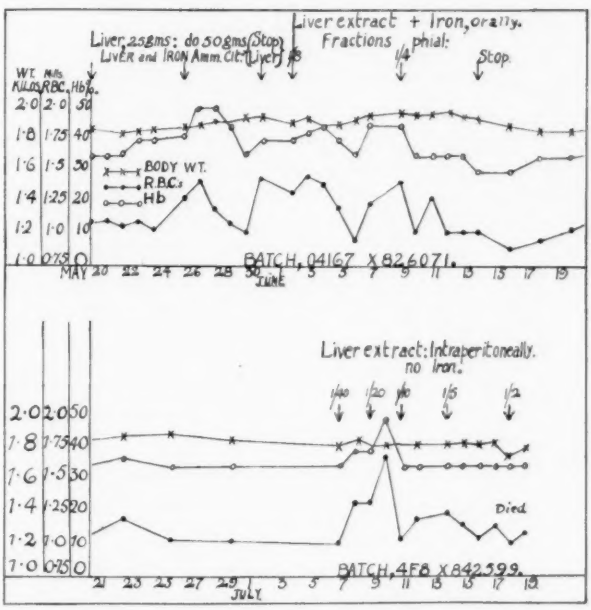

Chart 13.—Results in hen 384.

from the first part of the curves except that a rise of an irregular type, initiated by the liver, was maintained in a similar irregular fashion by the administration of liver extract. This was followed by a fall which persisted after stoppage of treatment on June 14. On July 7, liver extract was injected intraperitoneally with-

out the accompanying administration of iron by mouth; one-fortieth phial at once gave a marked response which persisted for a few days but subsequently fell, even although much larger doses of liver extract were administered.

The hen died on July 19. The peritoneal cavity was filled with the same brown material as in hens 377 and 378. The bones were markedly sclerosed and the marrow greatly diminished in quantity. The marrow was of a hyperplastic type.

Before the next series of observations was commenced, a summing up of the situation had been undertaken. It was recognized that, for some reason or other, as judged by the amount of liver extract necessary, the administration of this extract by mouth was much less efficacious in the fowl than in the human being. Intraperitoneal injection of a preliminary type had been resorted to with considerably better results. Such results, however, had been obtained in cases in which iron had been simultaneously administered by mouth. In a previous paper [1] it had been found that iron of itself was of no avail in ameliorating the disease. The results obtained in the present series of observations, in which the standard dose of iron was continued for long periods in conjunction with subeffective doses of liver extract without any rise in the curves, add confirmation to this observation. In the experiments heretofore the administration of iron had been continued as a routine on the chance that the possible absence of hemoglobin-forming materials might be a limiting factor in betterment of the blood and marrow condition, which the iron would help to rectify.

It was now decided to cease the administration of iron and to test the effect on the blood curves of injections intraperitoneally of the liver extract alone. It so happened, however, that the two first cases that occurred were of an identical and peculiarly interesting type. One of them was therefore employed as a bridging case to the new scheme, and this fowl (hen 387) was in consequence given iron orally.

The periods of the lives of fowls 377, 378 and 384 dealt with in the terminal portions of the respective charts (nos. 10, 11 and 13) are not included in this section, as these have to deal with the results of intraperitoneal injection of liver extract, without the conjoined administration of iron by mouth. It will be noted that in each there is a response in the curves to the dosage of one-fortieth phial.

Hen 387 (chart 14).—The administration intraperitoneally of one-fortieth phial of liver extract caused a rapid and extensive rise in the hemoglobin and red blood cell curves, which persisted under daily administration until the fowl was killed, on June 29.

The outstanding feature in this case is that the bones showed little or no sclerosis and consequently very slight diminution in the quantity of marrow. The marrow itself was hyperplastic in type. In addition, and as a characteristic feature, examination of the blood before treatment showed the presence of very large numbers of hemocytoblasts, plasma cell erythroblasts and erythroblasts. These observations indicated a very reactive condition of the marrow and almost

certainly are the explanation of the rapid and marked response shown in the chart. As the hemoglobin percentage and the number of red blood cells increased, there was a gradual disappearance of these immature cells until finally the cytology of the blood became practically normal.

Hen 389 (chart 15).—This case is almost identical with the last one, hen 387. There was, however, no administration of iron. As in hen 387, a marked reaction

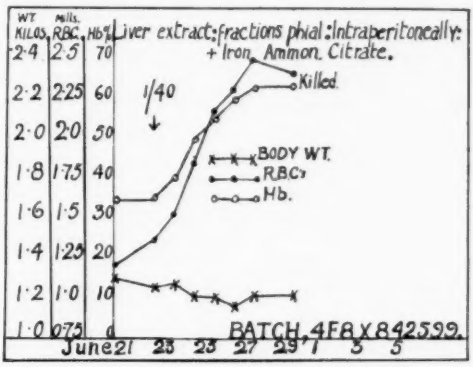

Chart 14.—Results in hen 387.

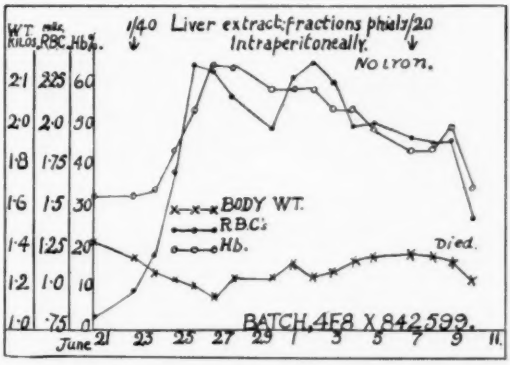

Chart 15.—Results in hen 389.

in both curves was obtained on the administration of one-fortieth phial of liver extract and on its continuance. After a time, on July 3, the effect began to wane and continued to do so, very little betterment being affected by increasing the dose to one-twentieth phial. The hen died on July 10. The immediate cause of death was a large hemorrhage from the liver. The postmortem observations and condition of the blood were exactly as described for hen 387.

Both cases would appear to be early ones, in contrast to the majority which were under observation. In them the marrow was in a very reactive and responsive condition and had not suffered much reduction

in quantity. Hen 317, discussed in a previous communication,[1] behaved similarly on the oral administration of liver extract and observations on its blood before treatment were of exactly the same nature. Such cases would appear to be ideal for the purpose of standardization of liver extract, but unfortunately their occurrence is the exception rather than the rule.

Hen 388 (chart 16).—Owing to the occurrence of holidays and to the fact that this fowl's blood was not considered to be in a sufficiently poor state for testing

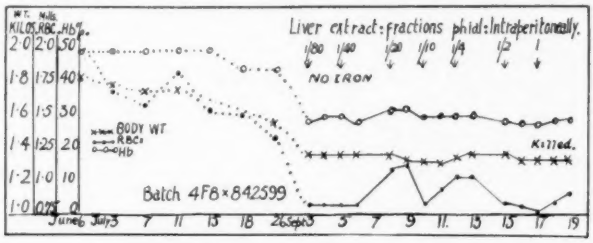

Chart 16.—Results in hen 388.

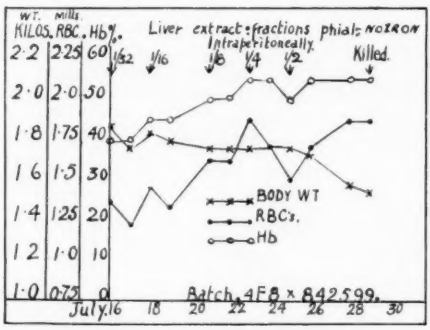

Chart 17.—Results in hen 395.

purposes, liver extract was not administered during the period covered by the first half of the chart. Observations on the blood were, however, made at intervals. When given, the liver extract was injected intraperitoneally without the accompaniment of iron. There is a definite response shown in the curves to the administration of one-fortieth phial. The reaction, however, is small and fleeting.

On postmortem examination the bones were found to be practically completely sclerosed, and marrow almost nonexistent. Any marrow that was present was in an aplastic state.

Hen 395 (chart 17).—Liver extract was administered intraperitoneally but no iron was given. A response occurred in both curves to a dose of one-thirty-second phial, and the rise continued with increasing dosage.

The hen was killed on July 29. The bones were markedly sclerosed and the marrow diminished in quantity. The marrow itself was in a hyperplastic state.

HEN 396 (chart 18).—The dosing was as in the previous case. A response occurred in both curves to a dose of one-thirty-second phial, and the rise was marked and progressive.

The fowl was killed on July 29. There was very little sclerosis of the bones, and the marrow was not much diminished in quality. It was in a hyperplastic state.

In this case there was exhibited, following the administration of liver, a marked rise in the number of hemacytoblasts, erythroblastic plasma cells and erythroblasts in the circulating blood. This condition reached its height about July 23. Thereafter the numbers diminished and the blood became practically normal cytologically. At the same time the hemoglobin and red blood cells increased.

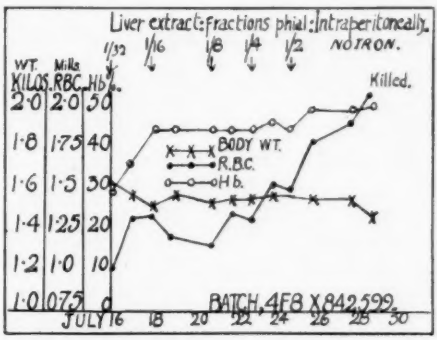

Chart 18.—Results in hen 396.

This phenomenon occurred to some extent in all the cases on the administration of liver extract. It is only, however, in cases with a reactive marrow that it reached proportions of sufficient dimensions to be correctly gaged. Owing to this limitation it cannot be used, like the analogous reticulocytosis in cases of human pernicious anemia, as an index for testing the potency of liver extract.

HEN 405 (chart 19).—The same dosing was employed as previously. A response occurred in both curves to a dose of one-fortieth phial, increasing thereafter with increasing dosage. Owing to some factor it was found impossible for a period to count the red blood cells, due to the rapidity with which the blood coagulated. The same phenomenon had been observed previously in an occasional fowl with pernicious anemia.

The hen was killed on October 27. There was considerable sclerosis of the bones and diminution of the bone marrow. The marrow itself was hyperplastic.

Although not specifically stated previously, every case dealt with here exhibited the postmortem appearances typical of primary or per-

nicious anemia of the fowl. In addition, several cases—especially among those in which liver extract had been given intraperitoneally for some time—showed considerable myelocytic proliferation in the periportal spaces of the liver. In some instances it reached a degree recalling the condition leukanemia. In quite a proportion of cases, too, the liver was studded all over with minute punctiform hemorrhages. These were found in great part to surround small periportal proliferations of myelocytes. Their significance is not understood. Under the conditions of their origin the administration of liver extract would appear to have something to do with their causation. In cases in which large doses of liver extract had been given intraperitoneally for long periods there was often an accumulation of turbid brown fluid containing inspissated brownish flakes in the peritoneal cavity. The brown

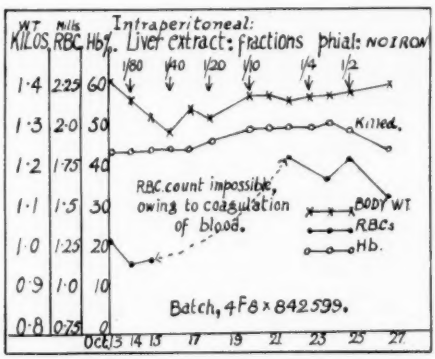

Chart 19.—Results in hen 405.

substance consisted in great part of necrosed cellular remains. It was striking, however, how rarely anything of the nature of an acute peritonitis occurred. When small doses were given for short periods, the peritoneal cavity was completely normal in appearance.

A survey of the cases described brings to view the fact that in practically none of the cases (cases 387, 389 and 396 are possible exceptions) did the hemoglobin percentage and number of red blood cells rise to normal on the administration of liver extract. It will be observed further that, after a preliminary rise to a maximum, there was in every case a subsequent marked fall, even although liver extract in increasing quantities continued to be given. Again, as it happened, many of the fowls died, and without doubt all would have done so in the long run, for a case of permanent recovery in this condition has never been observed.

In a previous paper,[1] reasons were suggested in the individual cases for the deaths of the experimental fowls even after they had been considerably benefited by the liver extract. It is now recognized that, while the reasons adduced on such occasions may have been of a contributory nature, without doubt the essential cause lies in the inherent perniciousness of the condition.

Pernicious anemia in fowls would seem to be, in every respect, a much more malignant and intractable condition than the corresponding disease in human beings. Its occurrence in fowls is also much greater, being in the neighborhood of 3 per cent per annum as compared with 0.005 per cent [6] in human beings.

The case histories given make it evident that the less bone sclerosis and the larger the amount of hyperplastic marrow present, the greater and more sustained is the improvement in the circulating blood. Furthermore, the cases so far observed show that the circulating blood gives evidence of a response on the part of the marrow to liver extract of a remarkably constant nature. Thus, the rise of the curves for the same extract in different fowls commenced with the same dosage, irrespective of the condition and amount of the marrow, provided only that it was in a hyperplastic state and not aplastic.

The changes occurring in the cytology of the circulating blood in relation to the amount and condition of the marrow have already been described. The resemblance of these phenomena to the reticulocytosis following the exhibition of liver extract in cases of human pernicious anemia has also been alluded to, and the difficulty of using them as an index for purposes of standardization has been referred to. Such observations and conclusions, arrived at some time ago, are supported by the recently published views of Riddle [7] on the relationship of liver feeding to reticulocytosis. He concluded, much as has been done in the present case, that the magnitude of the reticulocyte response is governed directly by the extent of hyperplasia of the bone marrow during relapse, and the rate of the reticulocyte response directly by the dosage of the active principle in liver.

These more or less preliminary points having been discussed, one may now turn to a consideration of the bearing of the observations on the institution of a method for determining the potency of liver extracts. In the light of later developments, the observations on liver extract, by feeding, may be now regarded as being of a preparatory nature and not worthy of discussion at any length. A point, however, may be singled out for notice. The minimum dose of the extract capable of eliciting a response when given to the diseased fowl by mouth would appear to be in the neighborhood of from one-fourth to one-half phial

6. McGowan: Brit. M. J. **2**:204, 1930.
7. Riddle, M. C.: Pernicious Anemia, Arch. Int. Med. **46**:417 (Sept.) 1930.

daily. Proportionately, therefore, to the dose required in man,[8] this is very large. As the extract, as will be discussed immediately, shows itself to be very active when injected intraperitoneally, it seems probable that the explanation of its weak action when given by mouth is that it is not easily absorbed [9] from the intestine of the fowl.

In discussing now the experiments with intraperitoneal inoculation, as the cases in which iron was administered in addition to the liver extract do not differ materially from those in which liver extract was used alone, there appears to be no need for considering them separately. A survey of the results, therefore, would seem to show that, in this case, the minimum dose of liver extracts potent in human cases (batches of liver extract 343, already mentioned) and capable of eliciting a response in the diseased fowl may be as low as (and may possibly be lower than) one-twentieth phial per day for white leghorn fowls of about 1,500 Gm. in weight, under the conditions already specified as regards environment, food, dosage, etc. This figure, however, must be regarded as only a rough and approximate one, regard being had to the stage of the investigation and to the essential and specific nature of the disease. It would seem best at present, moreover, to leave the results in this rough manner rather than to attempt to express them in the form of a possible fowl unit. An increased number of observations and improved technic will doubtless supply more accurate results. That, however, the accuracy obtained thus will not be available for the establishing of a unit such as exists, say, for insulin seems probable, the essentially different circumstances of the two cases being considered. The problem is also complicated in another way, for there are no figures available for the potency of liver extract when injected intraperitoneally or otherwise into human beings with pernicious anemia.

It is possible, however, that liver extract may be completely absorbed from the intestinal tract in human cases. Under such circumstances, comparison in regard to the efficacy of liver extract in human and fowl conditions will be possible. Lilly's liver extract is supplied in phials containing 4.5 Gm. For a human being weighing, say, 140 pounds (63. 5 Kg.), the recognized daily dose is about 3 phials or 13.5 Gm. The amount, therefore, of liver extract required per fourteen pounds of body weight would be 1.35 Gm. For a fowl, the corresponding figure, based on the present results, would be 0.875 Gm. When one considers that the 14 pounds and 3 phials are somewhat rough and arbitrary measurements, there is some justification, possibly slight, for considering the results obtained in the fowl and human being as being of the same order of magnitude.

8. The dose recommended and generally used in human cases is 3 phials per day.

9. It may possibly be destroyed in the intestine of the fowl.

The present mode of estimation takes no cognizance of the possibility that the response to the reactive dosage may be due to a cumulative effect of the previous subactive doses. Such a storage effect has been shown to exist in human beings by Cohn, McMeekin and Minot.[10] It is not easy to see how a method could be devised to get round this difficulty, which after all is not of real importance, all the circumstances considered.

The important question of the method of obtaining diseased birds for observational purposes now arises for discussion. In the present instance the experimental fowls were obtained as cases occurring sporadically in the ordinary course of events in an ordinary commercial flock. In this case the incidence of the disease has been such that during the last three years 65 cases have occurred in an annual population of 800 fowls under 2 years of age. This is equivalent to about 2.7 per cent, or roughly 3 per cent, a figure which has already been employed. For many purposes this may be too infrequent an occurrence, and the question arises whether the number of cases cannot be augmented.

It seems practically certain that the great majority of cases of pernicious anemia in the fowl are caused by infestation by the tapeworm, *Davainea proglottina*. It would be possible, therefore, to procure specimens by the feeding of fowls artificially with garden slugs, which act as the intermediate host and bearers of the cysticercoids of this tapeworm. Such experimental infestation has been carried out by Sawyer and Hamilton,[11] among others. This proceeding, however, would involve considerable labor and—as only a fraction of the fowls infected with *Davainea proglottina* develop the anemia—much disappointment. It would seem preferable, therefore, to employ some scheme based on what is known of the natural occurrence of this parasitic infection. The following would seem to meet the case. If fowls known to be hosts of *Davainea proglottina* were made to live with garden slugs in a confined area of a nature suitable (as regards the presence of vegetation, dampness, stones for protection, etc.) for the multiplication, development, etc., of the garden slug, this would seem to provide opportunities for intensified infestation. Under such circumstances, one would expect an increased supply of suitable cases of anemia.

CONCLUSIONS

A method for the quantitative determination of the potency of liver extracts by means of fowls suffering from pernicious anemia is described.

10. Cohn, McMeekin and Minot: J. Biol. Chem. **87**:IX, 1930.

11. Sawyer and Hamilton: West. Washington Station Bull. 10-W, 1928, p. 43.

In substance, it consists of the injection intraperitoneally, twice daily over a period of time, of increasing small doses of the extract until a dose is reached at which a "response" is obtained.

The response consists in a sharply appearing and definite increase in the hemoglobin percentage and number of red blood cells.

This response for the same sample of extract appears to be obtained at the same dosage, whatever the state of the marrow is, provided it is in a condition of hyperplasia and has not become aplastic.

The response dose for fowls under the conditions specified in the text is in the neighborhood of one-twentieth phial or 0.225 Gm. per day of the extracts employed.

A method is suggested for the procuring of a more abundant supply of experimental cases.

Dr. Clowes, director of the Eli Lilly Laboratories, furnished me with supplies of liver extract. Dr. Marion B. Richards prepared photographs and Mr. Archibald Macdonald, M.A., B.Sc., supplied me with experimental fowls.

ALBUMINURIA IN COLLEGE MEN* *

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C. A. McKINLAY, M.D.
MINNEAPOLIS

Every practicing physician has observed that albuminuria may occur in certain persons who in other respects seem perfectly normal. Concerning the significance and the frequence of this finding, many and diverse opinions have been expressed in a voluminous literature on the subject. Writers have described different types of albuminuria and have assigned to them such names as adolescent, orthostatic, postural, lordotic and cyclic. These terms doubtless were intended to be qualifying, descriptive and helpful, but their multiplication has seemed to complicate rather than to clarify the problem.

Of these descriptive terms the one most widely used is orthostatic albuminuria, a name suggested by Teissier,[1] who in 1899 commented on the relation of changes in posture to the appearance and disappearance of albumin in the urine of certain persons. In 1910, Jehle[2] published his monograph on this condition and suggested the further qualifying term of lordotic albuminuria. Many writers before and since that time have observed that other conditions, such as muscular exertion, emotional disturbances, increased body temperature, malnutrition, certain drugs, etc., also may produce albuminuria in healthy persons and in experimental animals (Goetsky,[3] Lewison, Freilich and Ragins[4]). Although the relationship of albuminuria to the erect, or orthostatic, position in certain cases and to lordotic posture in others has been definitely established, many persons with albuminuria show no evidence of lordosis, and many definitely crippled children do not show any higher percentage of albuminuria than children without lordosis. Furthermore, the difference in significance between these postural albuminurias and

* Submitted for publication, March 19, 1931.

* From the Student's Health Service, University of Minnesota.

* A preliminary report of this study was presented in the Kidney Symposium at the University of Minnesota in July, 1930, and is being published with the proceedings of this symposium.

1. Teissier, J. L.: Albuminurie de la station débout; albuminurie orthostatiques, Semaine méd. **19**:425, 1899.

2. Jehle, B.: Die lordatische Albuminuria, Berlin, Julius Springer, 1914.

3. Goetsky, F. K. H.: Zur Kenntnis der arthotischen Albuminuria, Diss., Berlin, 1910.

4. Lewison, M.; Freilich, E. B., and Ragins, O. B.: Lordosis as a Cause of Postural Albuminuria, Arch. Int. Med. **42**:440 (Sept.) 1928.

those that occur in other persons as a result of moderate exercise or emotional excitement or for no apparent cause is not clear. Hence in this report the use of the usual terms descriptive of types of albuminuria has been avoided.

This study of albuminuria in healthy young men is based on an analysis of the records of the physical examinations of 20,000 young men who entered the University of Minnesota as students between 1921 and 1930. In testing for albumin in the urine, the nitric acid test was used throughout, the reagent and the urine being layered in the test tube by means of a pipet.

INCIDENCE OF ALBUMINURIA

In this group of 20,000 young men, 1,065, or 5.32 per cent, were found to have albumin in the urine on the first examination, the amount of albumin varying from a trace, or one plus, in 79 per cent to a heavy cloud, three or four plus, in 6 per cent. Accurate comparison between these percentages and those reported by others is hardly possible, because the reagents that are used in performing tests for albumin differ in their ability to detect minimal amounts. Hill [5] reported that 27.5 per cent of 417 men in a citizens' training camp showed albumin on a single examination when heat and acetic acid or sulphosalicylic acid were used, while only 3.3 per cent showed albumin when nitric acid was used. Furthermore, the observed incidence of albuminuria in most groups would be increased if several specimens of urine instead of a single specimen were examined. For example, last year at the University of Minnesota the health examinations showed albuminuria in 93 upper classmen; of this number 22 per cent had had albuminuria at the time of their entrance examinations, while 78 per cent had been free from albumin in the urine at this time. In general, however, the reported incidence of albuminuria as shown by the nitric acid ring test performed on a single specimen of urine varies from 3 to 16 per cent, with from 5 to 7 per cent being the most common.

Reexamination of Students with Albuminuria.—Of these 1,065 students who showed albuminuria at the time of the entrance examination, 606 were reexamined by the Student's Health Service. Most of the others elected to go to private physicians for further study, while some dropped out of the university before the reexamination was made. It was the intention of the department to examine several specimens of urine, collected in the morning and in the evening, from each student who had shown albumin on the first examination, and in most cases this was done; a few students, however, failed to present additional specimens of urine for examination after having received one negative report. The results of these subsequent examinations are shown in table 1.

5. Hill, L. C.: Febrile Albuminuria, Quart. J. Med. **22**:305 (Jan.) 1929.

As will be seen from table 1, two thirds of the students who showed albuminuria at the time of the entrance examinations subsequently failed to show this condition; this is the group with so-called transitory albuminuria. Thirteen per cent had occasional albuminuria, most specimens being free from albumin, but more than one showing it. Twelve per cent had persistent albuminuria, all or nearly all specimens being positive for albumin, but without other evidences, at least convincing ones, of renal damage. Six and one-half per cent showed evidences of what was probably kidney disease. In this group with so-called "probably kidney disease" were placed those students who showed rather definite evidence of renal damage, as indicated by a considerable number or a

TABLE 1.—*Reclassification of Group with Albuminuria; Six Hundred and Six Students Reexamined*

Group	Percentage of Original Albuminuric Group	Percentage of Total Student Body (Calculated)
Transitory albuminuria	66.2	3.6
Occasional albuminuria	13.09	0.7
Persistent albuminuria	11.8	0.6
Probably kidney disease	6.5	0.3

TABLE 2.—*Quantity of Albumin in Relation to Its Persistence*

Quantity of Albumin on First Examination	Percentage of Final Classification		
	Transitory Albuminuria	Occasional or Persistent Albuminuria	Kidney Disease
Trace or +	74.6	25.2	2.1
++	52.9	36.8	10.3
+++	47.6	52.4	0.0
++++	6.3	75.0	18.7

persistence of leukocytes or blood cells or both in the sediment, the presence of a significant number of casts, a fixation of the specific gravity, inability on the part of the kidney to concentrate the urine or a diminished excretion of phenolsulphonphthalein. Most of the students in this group were studied by members of the Student's Health Service staff, but certain ones were under the care of private physicians, whose diagnoses of nephritis were accepted for this study.

The reclassification here used, of course, is an arbitrary one and only relatively accurate, for if a larger number of specimens had been examined, certain cases undoubtedly would have fallen into groups other than the ones in which they are now placed. The calculated percentages of the several classes of albuminuria show that in the student body as a whole albuminuria, except that which is transitory, is of infrequent occurrence.

PERSISTENCE OF ALBUMIN IN THE URINE

In this series there is a distinct relationship, as shown in table 2, between the amount of albumin reported on the first examination and its persistence on subsequent examinations; approximately three quarters of the students whose urines on entrance showed amounts of albumin reported as a trace or one plus later were classified as having transitory albuminuria, while only 6 per cent of those who had a four plus grade of albuminuria on the first examination were later free from albumin.

FACTORS RELATED TO THE OCCURRENCE OF ALBUMINURIA

As there is still considerable lack of information regarding the cause, the significance and the relationships of albuminuria in healthy persons, an analysis of certain physiologic data recorded for students with and without albuminuria has been made. For purposes of comparison in making this analysis, the following groups were set up:

1. The normal or control group, consisting of students in whose urines no albumin was discovered at the time of the entrance physical examination. Except for the last two years of the study, each one of the students in the control group was examined just before or just after a student with albuminuria. During the last two years the entire group of normals served as controls, but in computing the final percentages the results were weighed so as to have the same relation to the number of cases in the earlier years as obtained for the groups with albuminuria.

2. The group with transitory albuminuria, consisting of those students whose original examinations showed albuminuria, but whose urines on subsequent examinations were always free from albumin.

3. The group with persistent or occasional albuminuria, consisting of those students who were found on several occasions to have albuminuria and yet showed no definite evidence of kidney disease.

4. The group with kidney disease, previously described. Although an effort was made to exclude those whose cases were essentially vascular, the group probably includes students with various types of renal damage.

Age.—As shown in chart 1, the proportion of students who show albuminuria decreases progressively with age. Calvin, Isaacs and Meyer,[6] quoting Lauener, reported an increase in the incidence of albuminuria from childhood up to the age of 16 years. From this peak, which apparently occurs in the teens, there is a rapid decline in the incidence of the condition with advancing years to the group 30 years of age and over. The work of Sydenstricker and Britten[7] shows that

6. Calvin, J. K.; Isaacs, Bertha L., and Meyer, Jacob: Albuminuria in Children, J. A. M. A. **86**:182 (June 12) 1926.

7. Sydenstricker, Edgar; and Britten, R. H.: The Physical Impairments of Adult Life: General Results of a Statistical Study of Medical Examinations by the Life Extension Institute of 100,924 White Male Life Insurance Policy Holders Since 1921, Am. J. Hyg. **11**:73 (Jan.) 1930.

after the age of 40 an increase in the incidence of albuminuria again occurs, being progressive with age up to 70 and over.

Weight-Height-Age Percentage (chart 2).—The weight-height-age percentage here used represents the relationship of the individual student's weight to the standard, or average, weight for his age and height, as given in the medico-actuarial tables. The greater incidence of albuminuria among students of lighter weights is clearly shown in chart 2. The greater proportion of underweight students in the groups having the more persistent types of albuminuria and the smaller proportion of

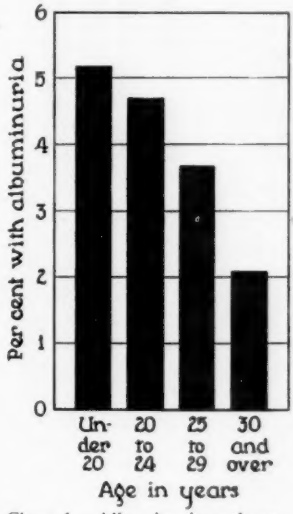

Chart 1.—Albuminuria and age.

overweight students in those groups are evidence of the close relationship of body build not only to the occurrence, but also to the persistence, of albumin in the urine.

Pulse Rate.—It is frequently stated that many persons who show albuminuria tend to be of a so-called high-strung, neurotic temperament. The striking increase in the incidence of albuminuria from 0.7 per cent in the students with pulse rates below 60 per minute to 18 per cent in those with pulse rates of 120 or over per minute lends remarkable support to this view. There is, however, no clear relationship between the pulse rate and the persistence of albumin in the urine. In fact, the groups with kidney disease and with persistent albuminuria show more uniform pulse rates than even the normal group.

Systolic Blood Pressure.—The percentage incidence of albuminuria is surprisingly uniform at the various levels of systolic blood pressure

(chart 4). There is also no tendency for hypertension to appear in any of the albuminuric groups except the group with kidney disease.

Diastolic Blood Pressure.—With the diastolic pressure, as with the systolic, there is no relationship between the level of the pressure

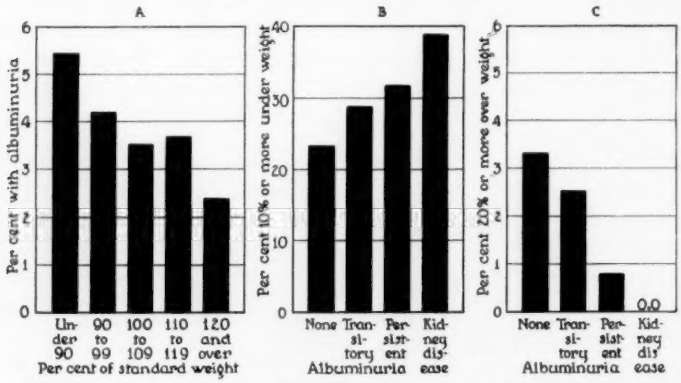

Chart 2.—Albuminuria and weight: *A*, incidence of albuminuria in various weight groups; *B*, underweights in the normal group and the several albuminuric groups; *C*, overweights in the normal group and the several albuminuric groups. In *A*, the standards used for weight, according to age and height, were taken from the medico-actuarial tables in general use.

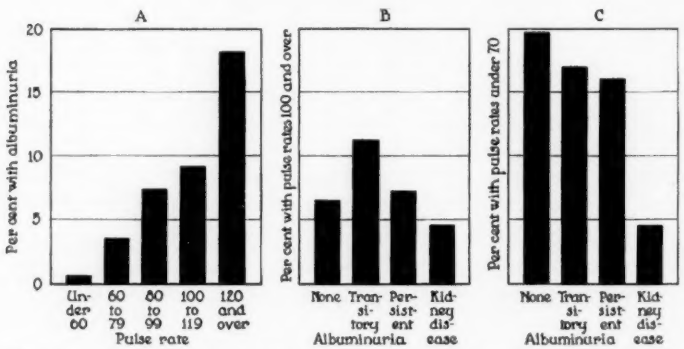

Chart 3.—Albuminuria and pulse rate: *A*, incidence of albuminuria in various pulse rate groups; *B*, rapid pulse rates in the normal group and the several albuminuric groups; *C*, slow pulse rates in the normal group and the several albuminuric groups.

and the incidence of albuminuria (chart 5). The increase of diastolic pressure in the group with kidney disease corresponds to the increase in systolic pressure. The progressively greater proportion of low diastolic

pressures in the several albuminuric groups suggests a relationship between albuminuria and the tonus of the circulatory system.

Scarlet Fever.—Past histories of those diseases thought most likely to be related to renal damage were compared for the normal group and the albuminuric groups. Scarlet fever (chart 6) had occurred with only a slightly greater frequency in the group with albuminuria than in the group without albuminuria. A greater percentage of students with the more persistent types of albuminuria gave histories of having had scarlet fever, but the differences between the groups were not sufficiently great to be statistically significant.

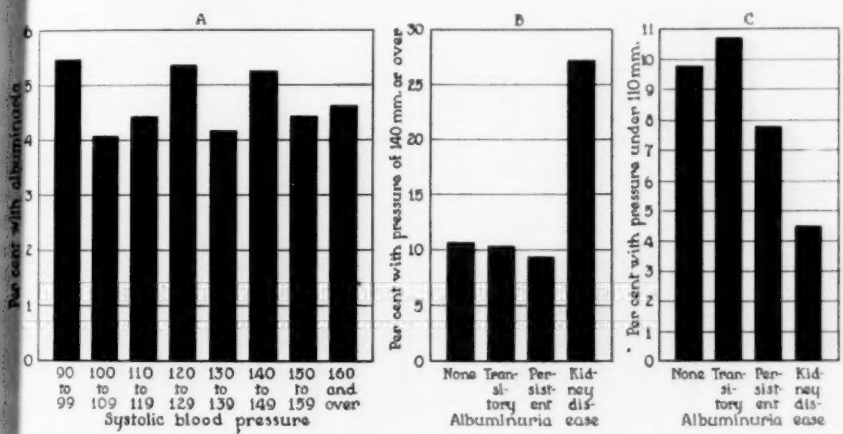

Chart 4.—Albuminuria and systolic blood pressure: *A*, incidence of albuminuria in various blood pressure groups; *B*, high systolic pressures in the normal group and the various albuminuric groups; *C*, low systolic pressures in the normal group and the several albuminuric groups.

Diphtheria.—Since diphtheria may cause a nephrosis, the incidence of diphtheria in the several albuminuric groups is compared in chart 6, but no relationship between these conditions is apparent.

Rheumatism.—Although a history of rheumatism without further qualification is inexact, the term rheumatism has been used for comparative purposes as indicative of rheumatic fever, absorption from a focus of infection or some similar condition. Although the history of this condition was given infrequently (chart 6), the incidence is greater in the group with albuminuria and there is a steplike increase in the incidence of rheumatism from the group with no albuminuria to those with transient and persistent albuminuria and renal damage. This relationship, although not frequently emphasized, is not surprising in view of the etiologic relationship between glomerular nephritis and rheumatic conditions.

Colds.—The entrance physical examination blank contains a question as to whether the student is subject to "frequent attacks of cold." Since the term "frequent attacks" is not accurately defined, there is a considerable possibility of inaccuracy in these replies, but in a large group they probably are fairly satisfactory for comparative purposes. As shown in chart 6, there is a significantly greater incidence of "frequent colds" among the students who have albumin in the urine than among those in the control group. There is also a distinct relationship between the persistence of albumin in the urine and the history of frequent colds. A possible reason for this relationship may be that many persons who are subject to repeated colds have chronic sinus infections with recurrent acute or subacute manifestations.

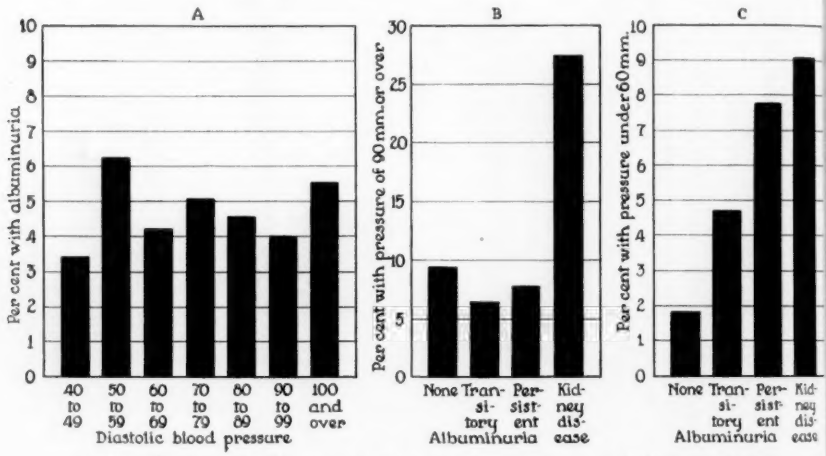

Chart 5.—Albuminuria and diastolic blood pressure: *A*, incidence of albuminuria in various blood pressure groups; *B*, high diastolic pressures in the normal group and the several albuminuric groups; *C*, low diastolic pressures in the normal group and the several albuminuric groups.

Tonsillitis.—The replies to the question on the history blank as to "frequent attacks of tonsillitis" are subject to the same inaccuracies and limitations as pertain to those concerning colds. The lack of relationship (chart 6) between albuminuria and a history of tonsillitis is surprising in view of clinical experience, but in this study it has been impossible to demonstrate any relationship.

Abnormal Tonsils.—The examinations of the nose and throat on which these records concerning the tonsils are based were made by otolaryngologists. Various terms were used by them to describe the conditions of the tonsils, but for the purpose of this analysis those

conditions that seemed to have pathologic significance were grouped together and called "abnormal tonsils." Chart 6 shows a slight relationship, although not a very significant one, between normal tonsils and the occurrence of albumin in the urine. There is, however, no relationship between the persistence of albumin and the condition of the tonsils. This agrees with Ashburn's [8] observation on West Point cadets, which he interprets as indicating that in his series there was

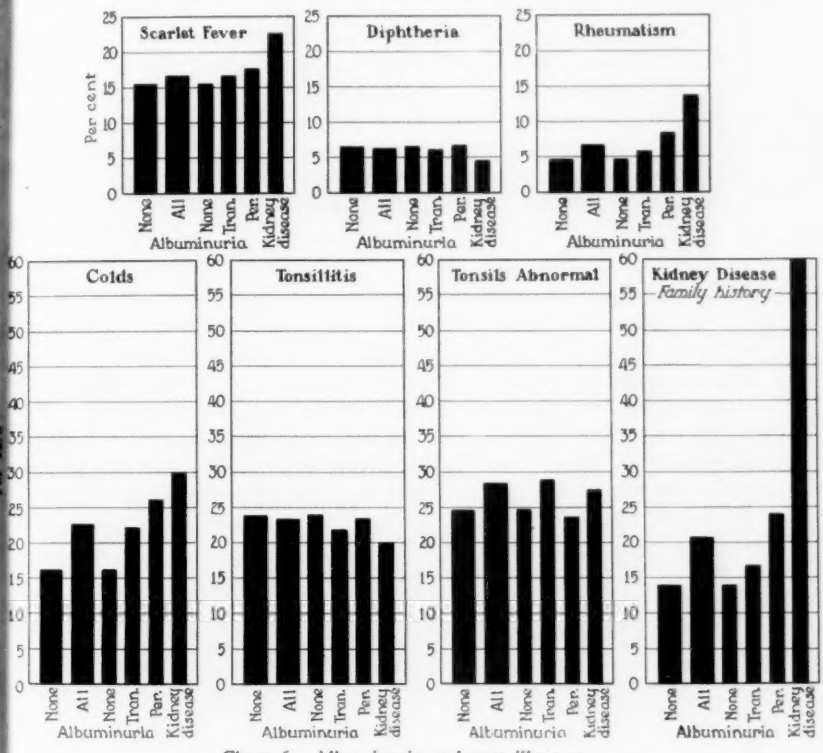

Chart 6.—Albuminuria and past illnesses.

nothing, not even in the results of tonsillectomies, to indicate that tonsillar infection was a causative factor in albuminuria.

Family History of Kidney Disease.—During the more recent years of this study, information concerning the family histories of various diseases has been obtained. This "family history" refers not only

8. Ashburn, P. M.: An Epidemic of Albuminuria, J. A. M. A. **90**:535 (Feb. 18) 1928.

to the immediate family, but also to grandparents and to uncles and aunts. One of the items about which inquiry is made in the family history is "kidney trouble." An analysis of these histories (chart 6) shows that students with albuminuria gave family histories of "kidney trouble" with distinctly and significantly greater frequency than the students who had no albuminuria. There is also a distinct, although not so positive, relationship between the persistence of albumin in the urine and the family history of "kidney trouble." This relationship between the family history of "kidney trouble" and albuminuria, although apparently quite definite for this group, might not be so striking if it were possible to eliminate from the group said to have kidney disease those adults whose disease was primarily vascular. However, we have found a less definite relationship between the blood pressures of the students and family histories of hypertension than between albuminuria and family histories of kidney disease.

COMMENT

The significance of albuminuria unassociated with other evidences of renal damage in young adults is an important question concerning which there is a distinct lack of agreement among physicians. Some writers believe that all or nearly all of the cases of albuminuria are, or have been, associated with some renal damage. Others believe that the condition is of little or no significance, and that in most persons albuminuria is consistent with the prospect of a perfectly normal life. This study, while not answering the all-important question as to the significance of albuminuria, shows certain interesting and significant relationships.

The slight statures, the rapid pulse rates, the lower diastolic blood pressures, the indications of a familial trait and even the greater susceptibility to infections among the students with albuminuria suggest that what certain writers have called a "constitutional inferiority" may be related to albuminuria in this group. In fact, after reviewing the evidence presented in the literature, Fishberg [9] concluded that "the constitutional factor is a very important one in determining the occurrence of albuminuria."

The higher blood pressure, both systolic and diastolic, in the group with kidney disease is just what would be expected. The slight, though hardly significant, upward tendency of the blood pressures of the other albuminuric groups could be caused by the inclusion in these groups of either some students with early primary hypertension or some with early renal damage, not yet recognizable by our methods of examination.

9. Fishberg, A. M.: Hypertension and Nephritis, Philadelphia, Lea & Febiger, 1930, p. 235.

As to possible contributory factors in the etiology of albuminuria, one finds a marked relationship between albuminuria, frequent attacks of "colds" and a family history of kidney disease; and a slight, though definite, relationship between albuminuria and a history of rheumatism or of scarlet fever. The possible interrelationship of these several conditions that seem related to albuminuria will be the subject of later investigation.

The definite steplike progression in the incidence of several of these conditions from the groups without albuminuria through those with transient and persistent albuminuria to the ones with kidney disease strongly suggests that either there are persons with unrecognized nephritis in these groups with transient, occasional and persistent albuminuria, or there are some physical or physiologic peculiarities in certain persons that tend toward the development of albuminuria and kidney disease. In either case it is doubtless well to be conservative in one's judgments as to the significance or lack of significance of occasional findings of albumin in the urine, even though with present methods one is unable to discover other evidences of renal damage.

SUMMARY

1. Of 20,000 male students at the University of Minnesota, 5.32 per cent showed albumin in the urine on the examination of a single specimen with the nitric acid ring test.

2. On reexamination in 606 cases of albuminuria, 66.2 per cent showed albumin on only one examination; 13.1 per cent showed occasional albuminuria; 11.8 per cent showed persistent albuminuria, and 6.5 per cent gave evidence of what was probably kidney disease.

3. The calculated percentage of the total group that showed persistent albuminuria and probably kidney disease is so small that even though there may be some overlapping in the groups, it is evident that kidney disease is a rare condition in young adults.

4. No significant relationships were found between albuminuria and systolic or diastolic blood pressure, a history of diphtheria or of tonsillitis or tonsils that on examination appeared abnormal.

5. Definitely positive and apparently significant relationships exist between albuminuria and weight, pulse rate, a history of rheumatism, "frequent colds" and a family history of kidney disease.

THE ACIDOSIS OF NEPHRITIS

ITS CLINICAL SIGNIFICANCE *

A. P. BRIGGS, M.D.
ST. LOUIS

In various types of chronic nephritis there develops a disturbance of the inorganic metabolism that is dependent, in part at least, on impairment of renal function. Sooner or later this disturbance is complicated to a variable degree by any or several of such concomitant disturbances as loss of appetite, vomiting, albuminuria and edema. On account of the discouraging number of variables encountered in clinical nephritis, Atchley and Benedict [1] studied the influence of obliteration of renal function in dogs by means of ligation of both ureters; they expressed the belief that they had produced the picture of uncomplicated renal insufficiency.

The plan of the present contribution was to study the inorganic disturbance in cases of clinical nephritis in which complications were relatively slight. Observations were made over a period of three years during intervals when disturbing influences for the most part seemed negligible. Appetite was not infrequently poor, but there was no vomiting except as stated. Although albuminuria was present to a variable degree, the serum protein was maintained above 5 per cent, and edema, if present on the patient's entrance to the hospital, had responded to treatment for an underlying cardiac disturbance.

CLINICAL MATERIAL

The type of renal disease in most of the cases studied was arteriosclerotic nephritis, according to the classification of Addis.[2] The majority of the patients were middle aged or elderly persons with a history of shortness of breath and swelling of the ankles or lower extremities appearing shortly before entrance to the hospital. Examination in this group revealed hypertension, albuminuric retinitis and signs of heart failure. With rest in bed and moderate doses of digitalis there were prompt symptomatic improvement and disappearance of edema.

* Submitted for publication, March 16, 1931.

* From the Department of Internal Medicine, St. Louis University School of Medicine.

1. Atchley, Dana W., and Benedict, Ethel M.: The Distribution of Electrolytes in Dogs Following Ligation of Both Ureters, J. Biol. Chem. **73**:1 (May) 1927.

2. Addis, T.: The Renal Lesion in Bright's Disease, Am. J. M. Sc. **176**:617 (Nov.) 1928.

The patient in case 66997 was nauseated and vomited occasionally on the day of entrance, influences of which are seen in the analysis of serum obtained the following day. He died a few days later. In case 67676 there had been some nausea and vomiting associated with an infection of the upper respiratory tract a few days before blood was taken for analysis.

In cases 67156 and 68497 the patients were younger persons with high blood pressure and similarly advanced renal impairment. The former patient had marked thickening of palpable arteries and advanced retinal sclerosis; the latter had no perceptible thickening of palpable arteries and only beginning retinal sclerosis. These findings were of special interest in connection with the difference in the tolerance of the patients for mineral acid and the question of uremia. In the latter case there was a questionable diagnosis of hemorrhagic nephritis on account of persistent slight hematuria.

Case 67643, that of a woman aged 22, was one of rapidly progressing malignant hypertension.

Case 65255 was one of subacute nephritis with hematuria and slight edema.

METHODS

Except during metabolism tests or when otherwise indicated, the subjects received the regular house diet.

Blood was taken for analysis about four hours after the morning meal. From 30 to 40 cc. was drawn with minimal stasis into an oil-coated syringe, and discharged under oil into a 50 cc. centrifuge tube. The carbon dioxide content was determined promptly after centrifugation with an original Van Slyke apparatus. The serum was then removed with a pipet. Duplicate samples of 1 cc. each were measured into 50 cc. pyrex beakers for determination of the total base; a 0.5 cc. sample was transferred to a 50 cc. volumetric flask for determination of protein nitrogen; 2 cc. was saved for emergency; the rest was precipitated with trichloracetic acid for determination of phosphoric, sulphuric and hydrochloric acids and of nonprotein nitrogen. In a few instances samples of serum were taken before exposure to air for colorimetric determination of p_H. Determinations of the various acids were not made in duplicate and were repeated only in case of accident or doubtful result.

Urine was collected in large stoppered bottles containing chloroform as a preservative.

Chemical Technic.—The total fixed base was determined for serum by a technic essentially that of Stadie and Ross.[3] It was observed that results were low if any appreciable amount of carbon remained after evaporating the digest to dryness, presumably because some sulphate was converted to oxide and part of this to silicate during the ignition, and also that numerous additions of nitric acid were required to obtain complete digestion when the procedure was carried out as directed by Stadie. Accordingly, the following technic, which gave good digestion and required little attention, was adopted: To the sample of serum, 0.5 cc. of

3. Stadie, William C., and Ross, Effie C.: A Micro-Method for the Determination of Base in Blood and Serum and Other Biological Materials, J. Biol. Chem. **65**:735 (Oct.) 1925.

sulphuric acid and 10 cc. of nitric acid were added; the beaker was covered with a watch glass, and the mixture was boiled gently for six hours. The cover glass was then removed, the digest evaporated to dryness, and the determination completed according to the Stadie technic. Addition of potassium dihydrogen phosphate in quantities up to the equivalent of 0.1 mg. of phosphorus to serum or to samples of solutions of known fixed base had no influence on the result. Phosphorus was, therefore, not removed from serum, but the correction for base bound as metaphosphate in the ignited residue was calculated from the phosphate determination. The value given for serum fixed base is the average of duplicate determinations that checked within 4 milliequivalents. Fixed base in urine was determined by a similar technic after removal of phosphate with ferric iron. Serum chloride, in the work on case 68497, was determined by the method of Wilson,[4] and in the other cases by a similar titration on trichloracetic filtrate. Chloride in urine was determined as in the Wilson method. Sulphate in urine was determined by a benzidine titration as in the method of Fiske.[5] However, phosphate was removed by centrifugation after precipitation with calcium acetate, and the benzidine sulphate was likewise separated and washed by centrifugation. Sulphate in trichloracetic filtrate was precipitated as in the method of Wakefield,[6] but the color used for comparison was that of Yoshimatsu.[7] In my hands this color, developed with ammonia and iodine, has proved to be more intense and stable and more quickly developed than the color developed with peroxide and ferric iron which was used by Wakefield. In the earlier part of this study, serum sulphate was determined by the method of Denis and Reed.[8] The phosphate in trichloracetic filtrate and urine was determined colorimetrically as in the Briggs method.[9] The reducing agent employed was a solution containing 1 per cent hydroquinone and 5 per cent sodium bisulphite. For each 0.1 mg. of phosphorus anticipated 1 cc. of the reducing agent was added. A valence of 1.8 was assumed for phosphate in both serum and urine as in the study of Gamble and his associates.[10] Serum base bound as bicarbonate was calculated by means of the factor 0.423, used by Atchley and Benedict.[1] The method used for the determination of sodium in urine was that of Kramer and Gittleman.[11] Ammonia in urine was determined by the permutit

4. Wilson, D. Wright, and Ball, Eric G.: A Study of the Estimation of Chloride in Blood and Serum, J. Biol. Chem. **79**:221 (Sept.) 1928.

5. Fiske, Cyrus H.: The Determination of Inorganic Sulfate, Total Sulfate, and Total Sulfur in Urine by the Benzidine Method, J. Biol. Chem. **47**:59 (June) 1921.

6. Wakefield, E. G.: The Colorimetric Determination of Total and Inorganic Sulfates in Blood Serum, Urine, and Other Body Fluids, J. Biol. Chem. **81**:713 (March) 1929.

7. Yoshimatsu, S.: Colorimetric Methods for Determining Sulfate in Urine, Blood, and Milk, Tohoku J. Exper. Med. **7**:119 (April) 1926.

8. Denis, W., and Reed, Lucille: Methods for the Determination of Some of the Non-Protein Sulfur Compounds of Blood, J. Biol. Chem. **71**:191 (Dec.) 1926.

9. Briggs, A. P.: A Modification of the Bell-Doisy Phosphate Method, J. Biol. Chem. **53**:13 (July) 1922.

10. Gamble, James L.; Blackfan, Kenneth D., and Hamilton, Bengt: A Study of the Diuretic Action of Acid Producing Salts, J. Clin. Investigation **1**:359 (April) 1925.

11. Kramer, Benjamin; and Gittleman, I.: An Iodometric Method for the Determination of Sodium in Small Amounts of Serum, J. Biol. Chem. **62**:353 (Dec.) 1925.

method of Folin and Bell.[12] Proximate p_H determinations were made on urine as directed by Clark,[13] and on serum by the method of Cullen.[14] Protein nitrogen in serum was determined colorimetrically, acacia being used for a protective colloid as suggested by Chiles.[15] Nonprotein nitrogen in trichloracetic filtrate was determined similarly. The factor 2.36 was used as by Atchley and Benedict[16] for calculating base bound by protein. Creatinine in urine was determined by the Folin method.[17]

SERUM ACID AND BASE

The condition of the electrolytes, as shown in table 1, is seen to be almost similar to that observed by Atchley and Benedict[1] in the early stage of kidney block produced by ligation of both ureters. The levels of phosphate and sulphate are elevated, and this change is approximately balanced by a drop in bicarbonate with no characteristic change in chloride. The level of serum fixed base in these patients, however, is slightly lower than the average normal value which was unchanged by experimental kidney block in the study of Atchley and Benedict.[1]

Studies of unselected cases of clinical nephritis usually show wide fluctuations in the level of chloride, with low values more common than high. Inspection of the tables and protocols of the studies of Bulger, Peters and their co-workers[18] and Hartmann and Darrow[19] and of those of the study of Atchley and Benedict,[16] which has just appeared,

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TABLE 1.—*Condition of Electrolytes in Blood Serum in Nephritis* *

Case and Date	NPN	p_H	HCO_3	PO_4	SO_4	Cl	Protein	Total Acid	Total Base	Difference Between Base and Acid	Remarks
M. P. 62367											
2/ 1/1928	91	....	18.1	3.6	3.0	102.5	14.8	142.0	146.1	4.1	
2/11/1928	89	....	21.6	3.8	2.7	100.0	14.9	142.9	145.9	3.0	High base diet
2/24/1928	93	....	20.3	2.6	3.7	102.6	14.4	143.6	148.5	4.9	$CaCO_3$, 6 Gm. daily since 2/11/1928
L. C. 64392											
12/ 4/1928	67	....	19.9	3.7	1.6	104.1	14.9	144.2	150.2	6.0	
12/29/1928	67	....	22.7	2.5	1.0	105.0	14.9	146.5	150.0	3.1	$SrCO_3$, 6 Gm. daily since 12/4/1928
1/19/1929	...	....	20.3	3.6	2.2	102.6	15.3	144.0	147.3	3.3	
2/ 4/1929	72	....	18.1	5.6	1.5	100.0	15.2	140.4	145.4	5.0	H_3PO_4 test
B. J. 64838											
2/26/1929	105	....	17.5	4.4	4.6	104.8	14.6	145.9	150.4	4.5	
3/ 4/1929	110	....	18.6	3.1	4.3	105.9	14.6	146.5	150.7	4.2	$CaCO_3$, 6 Gm. daily since 2/26/1929
3/ 9/1929	125	....	13.9	5.8	5.2	102.5	15.2	142.6	147.1	4.5	H_3PO_4 test
E. S. 65255											
5/27/1929	52	....	24.1	2.6	1.2	102.5	15.3	145.7	148.5	2.8	
6/10/1929	...	....	22.8	2.4	1.3	105.0	15.5	147.0	152.4	5.4	
J. V. 66997											
2/20/1930	121	....	19.5	5.0	5.3	98.0	12.8	140.6	143.8	3.2	Recent vomiting
M. P. 66998											
3/29/1930	158	....	14.8	5.2	6.6	102.0	14.3	142.9	146.1	3.2	
4/21/1930	154	....	18.6	1.9	3.6	102.4	16.2	142.7	147.5	4.8	$SrCO_3$, 6 Gm. daily since 3/29/1930
5/ 7/1930	150	....	18.6	2.9	4.0	104.0	13.6	143.3	148.6	5.3	High base diet
E. D. 67024											
2/24/1930	105	....	17.6	3.2	3.4	104.1	14.9	143.2	148.5	5.3	
3/13/1930	97	....	22.2	3.1	2.9	102.3	13.6	144.1	147.8	3.7	High base diet
3/16/1930	...	....	14.1	2.7	7.2	103.0	14.7	141.7	147.0	5.3	$(NH_4)_2$ SO_4 test
4/ 1/1930	100	....	18.7	4.2	3.6	102.3	14.3	142.1	147.5	5.4	
J. L. 67156											
3/19/1930	125	7.36	16.3	4.4	5.0	102.3	15.8	143.8	150.4	6.6	
3/22/1930	124	7.24	13.3	7.0	5.2	102.0	15.3	142.7	145.9	3.2	H_3PO_4 test
4/25/1930	103	7.38	18.2	2.8	3.5	106.8	15.6	146.9	149.5	2.6	$SrCO_3$, 6 Gm. daily since 3/22/1930
5/14/1930	125	....	17.4	3.9	4.7	104.9	15.2	146.1	150.9	4.8	
5/26/1930	167	7.28	13.9	4.8	6.6	108.2	16.6	150.1	154.7	4.6	Liver administration
6/12/1930	130	....	16.1	3.5	5.7	104.1	14.7	144.1	148.9	4.8	
D. H. 67506											
5/15/1930	62	....	18.6	3.1	1.2	103.6	16.8	145.4	148.7	3.3	
6/ 3/1930	...	7.40	20.7	3.1	1.2	105.0	16.4	147.1	150.7	3.6	
6/18/1930	59	7.28	16.1	2.8	5.0	107.1	17.1	148.0	151.6	3.6	$(NH_4)_2$ SO_4 test
L. S. 67643											
6/12/1930	66	....	22.8	3.0	2.0	99.3	15.7	142.8	146.1	3.3	Recent vomiting
J. S. 67676											
8/ 2/1930	66	....	21.4	3.2	1.2	100.8	13.8	140.4	146.6	6.2	Recent vomiting
E. M. 67985											
8/13/1930	52	....	25.8	2.5	0.9	104.0	16.8	150.0	155.4	5.4	
8/22/1930	...	....	21.0	2.5	1.7	105.8	16.8	148.8	151.8	3.0	$(NH_4)_2$ SO_4 test
G. F. 68497											
10/ 9/1930	119	7.38	20.2	3.3	4.8	107.5	15.6	151.4	153.8	2.2	
10/15/1930	121	7.20	14.5	3.7	9.2	104.7	18.3	150.4	154.0	3.6	$(NH_4)_2$ SO_4 test
11/15/1930	120	....	19.9	3.4	4.6	101.2	15.6	144.7	149.8	5.1	
12/20/1930	118	....	21.0	2.2	2.1	104.0	16.1	145.4	150.2	4.8	$SrCO_3$, 6 Gm. daily since 11/15/1930
12/29/1930	108	....	21.4	3.8	4.0	99.1	15.5	143.8	145.5	1.7	Recent vomiting
1/ 8/1931	111	....	20.7	4.1	3.8	104.5	15.4	148.5	152.3	3.8	

* The figures for acids and bases represent milliequivalents per liter. Average values obtained by the same methods on patients without disturbance of inorganic metabolism are approximately as follows: $HCO_3 = 27$, $Cl = 105$, $PO_4 = 1.8$, $SO_4 = 0.5$, protein = 17.6, and total fixed base = 152.

shows an occasional level of chloride somewhat higher than normal, but associated with edema, a complication that was negligible in the present study. There is rather common association of a low chloride level with vomiting or with dehydration resulting from vomiting and diarrhea. In many of the reported cases with advanced functional impairment but without vomiting or edema, the chloride values were strictly normal, like those of the present study. It seems doubtful, then, that abnormal serum chloride values are characteristic of the disturbance resulting from impaired renal function.

There is a clear relation of phosphate and sulphate retention to renal insufficiency, as pointed out by Atchley and Benedict.[16] The level for serum protein is frequently lower than in the present study, but when low is associated with edema. The question of ketone acid retention raised by Peters and his co-workers [18] seems to have been answered in the study of Atchley and Benedict.[16] In the present study there was no occasion to suspect a starvation or pancreatic ketosis, and it will be observed that the balance obtained between total acids and fixed base usually leaves little room for accumulated organic acid.

It should be added that the observations considered so far were fairly well indicated in the early study of Denis and Hobson,[20] who showed rising levels of sulphate and phosphate and depletion of the alkaline reserve associated with retention of nonprotein nitrogen and creatinine. A few high chloride values were associated with edema; the few low chloride values were obtained from moribund patients with a presumptive association of vomiting.

In the study of Denis and Hobson [20] the basic elements were determined separately; on account of inherent errors in the sodium method, the values for total fixed base, calculated from their results, probably are all too high. Still, except with patients in a moribund state and with chloride levels suggestive of vomiting, the values obtained for the basic elements were similar to those obtained for normal controls by the same methods. Fixed base depletion was not considered by these authors as a characteristic of advanced clinical nephritis. Subsequent work with improved technic has likewise failed to demonstrate serious depletion of serum fixed base to be such a characteristic. The studies of Peters et al.,[18] and of Atchley and Benedict [16] showed marked depression of the fixed base level to be consistently associated with vomiting, and in the study of Hartmann and Darrow [19] the conspicuously low levels of fixed base were associated with dehydration due to vomiting and diarrhea. All of these authors

20. Denis, W., and Hobson, S.: A Study of the Inorganic Constituents of the Blood Serum in Nephritis, J. Biol. Chem. **55**:183 (Feb.) 1923.

observed, however, that in cases in which extraneous causes for loss of fixed base appeared to be absent, the level of serum fixed base in chronic nephritis with functional impairment was usually at or slightly below the lower limit of normal fluctuation; they have concluded that this was due to some defect in the renal mechanism for the conservation of fixed base.

In the present study, in which complicating disturbances were minimum, the level of serum fixed base was observed to be usually between 145 and 152 milliequivalents per liter, whereas the normal limits obtained by Atchley and Benedict [16] were from 148 to 155. The levels in health and chronic nephritis are, therefore, so close together that their fluctuations overlap. About 1 milliequivalent of this discrepancy can be accounted for by the influence of a high phosphate and low protein content on serum calcium. A small balance of the discrepancy remains, which it seems might well be due to some renal defect in conservation of base. Much of the present study was concerned with the nature of this defect.

URINARY EXCRETION OF INORGANIC CONSTITUENTS

Examination of the urine in nephritis fails to show insufficient excretion of the inorganic acids. Boyd [21] found that nephritic and normal children on the same diet excreted similar quantities of phosphoric acid. Gamble and his associates [10] found that a nephritic and a normal child on the same diet excreted similar quantities of the various acids and total fixed base. Van Slyke and his co-workers [22] found the titratable acid of the urine well maintained in nephritis until the terminal stage. Apparently the nonthreshold acids, i. e., sulphuric and phosphoric, accumulate in the blood to an equilibrium level at which filtration or secretion per glomerulotubular unit of kidney remnant is sufficiently increased to maintain metabolic balance.

In considering the excretion of fixed base in nephritis we are not so much concerned with elimination as with conservation. Excess of any of the basic elements is thrown off by the intestine as well as by the kidney, so that retention of fixed base is not a problem in renal insufficiency. The question of conservation of fixed base, on the other hand, is of much interest, since many foodstuffs in the average diet contain more potential acid than base, i. e., have an acid ash

21. Boyd, Gladys L.; Courtney, Angelia M., and MacLachlan, Ida F.: The Metabolism of Salts in Nephritis: I. Calcium and Phosphorus, Am. J. Dis. Child. **32**:29 (July) 1926.

22. Van Slyke, D. D.; Linder, G. C.; Hiller, Alma; Leiter, L., and McIntosh, J. F.: The Excretion of Ammonia and Titratable Acid in Nephritis, J. Clin. Investigation **2**:255 (Feb.) 1926.

(Sherman and Gettler [23]), and the kidney carries the burden of excreting this excess acid with a minimum loss of fixed base.

It is simple enough to suppose that the pathologic condition observed in usual types of chronic nephritis with renal insufficiency might lead to retention of the less diffusible acids, which depend on the kidney for excretion; but it is not so easy to understand why this same condition should lead to a waste of fixed base. That is to say, in view of the prevailing conceptions of renal pathology and physiology, if the damage to the kidney is primarily at the glomerulus, whether from a toxin of hemorrhagic nephritis or from the process of arteriosclerosis, one might expect, with decreased formation of glomerular fluid, as indicated by the studies of Rehberg,[24] higher levels of various urinary constituents in the blood stream. On the other hand, if damage to the tubule is the result of occlusion of the glomerular tuft, the nutrition and ability of any tubule to reabsorb vital constituents should be preserved in proportion to the circulation through the corresponding glomerulus and somewhat in proportion to the amount of glomerular fluid formed.

During the course of this study it has become obvious that many phases of the inorganic disturbance in nephritis are made clearer by a consideration of the probable difference between a nephritic and a normal subject with regard to the rate of flow of glomerular fluid through the tubules. Marshall [25] and Carr [26] have shown that diuresis produced by drinking distilled water makes the urine more alkaline. Excretion of inorganic acids is increased, but excretion of ammonia is unchanged. Diuresis, therefore, causes waste of more fixed base than acid. Apparently the increased rate of flow of glomerular fluid through the tubules affords less opportunity for modification by reabsorption of fixed base or enrichment with acid. The urine more closely resembles plasma in composition. In any of the ordinary types of chronic nephritis with impaired renal function the volume of urine is somewhat greater than normal, especially at night. The situation is, therefore, one in which an increased volume of urine passes through

23. Sherman, H. C., and Gettler, A. O.: The Balance of Acid-forming and Base-forming Elements in Foods and Its Relation to Ammonia Metabolism, J. Biol. Chem. **11**:323, 1912.

24. Rehberg, Poul Brandt: Ueber die Bestimmung der Menge des Glomerulus-Filtrats mittels Kreatinin als Nierenfunktionsprüfung, nebst einigen Bemerkungen über die Theorien der Harnbereitung, Zentralbl. f. inn. Med. **50**:367 (April 13) 1929.

25. Marshall, E. K., Jr.: The Influence of Diuresis on the Elimination of Urea, Creatinine and Chlorides, J. Pharmacol. & Exper. Therap. **16**:141 (Oct.) 1920.

26. Carr, A. D.: The Effect of Water Diuresis on the Elimination of Certain Urinary Constituents, J. Pharmacol. & Exper. Therap. **18**:221 (Oct.) 1921.

relatively few remaining tubules. The rate of flow through the tubules must be considerably increased. Starting at the other end of the process, it may be supposed that in each surviving renal unit a compensatory increased rate of formation of glomerular fluid occurs, although the total quantity of glomerular fluid as calculated by Rehberg [24] is decreased. With increased rate of flow through the tubules, imperfect reabsorption of water and fixed base and, to a lesser extent, of chloride and other constituents of the glomerular fluid results. Since this compensatory diuresis is sustained day and night, evidence of slight depletion of fixed base in serum seems to be adequately accounted for.[27]

Hartmann and Darrow [19] suggested that the polyuria of chronic nephritis might in some way be related to the reduced level of serum fixed base. Atchley and Benedict [16] expressed the belief that such a view lacks experimental proof, and pointed to the failure of diuresis to change the level of serum fixed base in a patient with cardiac disease and edema and good renal function. They observed later, nevertheless, that the fixed base level in a nephritic patient was reduced 5 milliequivalents by forcing fluids for one week. This result might be anticipated by a consideration of the probable difference in the rate of flow through the tubules of a patient with cardiac disease and those of a nephritic patient, for an increase in the volume of urine of any magnitude distributed among the hundreds of thousands of tubules of a normal renal apparatus would not be expected to create nearly so great an increased rate of flow as that produced by the few glomerulotubular units of a kidney remnant. A diuresis that caused excretion of little extra fixed base in the normal renal apparatus might result in the loss of a considerable quantity in the nephritic patient. Experimental support for this assertion is offered in the study of case 68497, table 7.

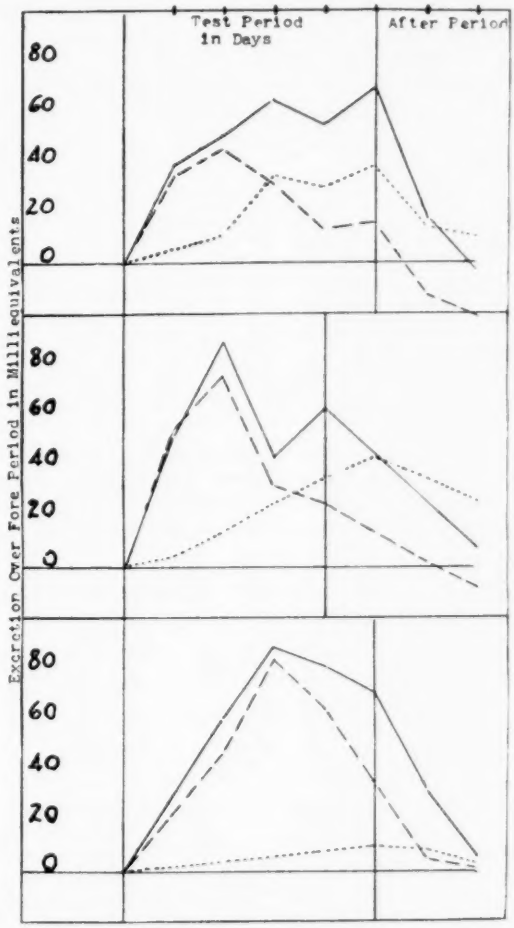
Certain observations of Gamble and his associates [10] and Linder [28] should be considered at this point. They have shown that administration of mineral acid leads to the excretion of more extra fixed base in the urine of a subject with impaired kidney function than in that of normal controls. The results of these studies have led to the conclusion that a specific defect in the process of conservation of fixed base exists in the nephritic kidney, and that this defect is intimately connected with the process of ammonia formation, which is proportionately depressed.

In order to study this question further, a number of metabolism experiments similar to those cited have been conducted, and the results

27. A demonstrable lowering of serum chloride could hardly be expected, since diuresis leads to waste of less chloride than of fixed base and the depression of the level of fixed base is very slight.

28. Linder, Geoffry C.: The Effect of Mineral Acid on Acid-Base Regulation in Health and in Nephritis, Quart. J. Med. **20**:285 (April) 1927.

of three of them, in which cooperation of the subject was satisfactory in regard to collection of urine and adherence to diet, are shown in tables 4, 5 and 6. The results of these studies on nephritic patients without edema are compared graphically in the chart with those on a



Increased excretion of acids and bases in urine on administration of ammonium sulphate. The upper part of the chart represents the results in a subject with normal renal function (case 67075, table 2); the middle part, those in a case of nephritis with edema (A. T., table 3), and the lower part, those in a case of advanced nephritis without edema (case 68497, table 6). The excretion of mineral acid is indicated by a solid line, that of fixed base by a long dash and that of ammonia by a short dash.

normal control (table 2) and on the nephritic patient with edema studied by Gamble and his associates [10] (table 3).

It will be observed that in nephritis without edema there is no more waste of base during the first two days of the study than in the normal control and a trifle less than in nephritis with edema. During the last part of the acid test period the waste of base in case 68497, in which

TABLE 2.—*Case 67075. Normal Renal Function. Influence of Ammonium Sulphate on Excretion of Acids and Bases in Urine*

Day	$(NH_4)_2SO_4$ Ingested, M.Eq.	Urine Volume, Cc.	Creatinine, Gm.	p_H	SO_4, M.Eq.	Cl, M.Eq.	PO_4, M.Eq.	Mineral Acid, M.Eq.	Na, M.Eq.	Fixed Base, M.Eq.	NH_4, M.Eq.	Increase Over Fore Period: Mineral Acid	Fixed Base	NH_4
1	0	960	0.81	5.8	42.4	34.1	32.0	108.5	42.6	88.3	18.4	....	....	....
2	0	1050	0.88	5.8	47.3	35.3	29.2	111.8	51.7	92.6	20.2	....	....	....
3	0	1120	0.92	5.6	48.7	37.1	34.4	120.2	47.6	95.1	21.7	....	....	....
4	75	1080	0.81	5.4	85.5	35.3	32.6	153.2	62.3	127.5	26.2	39.7	35.5	6.1
5	75	1160	0.89	5.0	94.5	40.5	30.1	165.1	78.7	132.4	31.5	51.6	42.4	11.4
6	75	1320	0.90	5.0	100.2	42.8	35.2	178.2	58.2	120.0	34.5	64.7	28.0	34.4
7	75	1010	0.82	5.2	98.6	40.6	29.8	169.0	49.7	105.3	50.6	55.5	13.3	30.5
8	75	1230	0.86	5.2	106.2	41.3	34.4	181.9	51.6	108.0	59.5	68.4	16.0	39.4
9	0	980	0.81	5.4	61.3	40.5	28.5	130.3	....	79.2	39.4	16.8	—12.8	17.3
10	0	920	0.83	5.6	49.5	31.6	29.0	110.1	....	71.4	31.5	— 3.4	—20.6	11.4

TABLE 3.—*Case A. T. Nephritis with Edema.* Influence of Ammonium Sulphate on Excretion of Acids and Bases in Urine*

Day	$(NH_4)_2SO_4$ Ingested, M.Eq.	Urine Volume, Cc.	Creatinine, Gm.	p_H	SO_4, M.Eq.	Cl, M.Eq.	PO_4, M.Eq.	Mineral Acid, M.Eq.	Fixed Base, M.Eq.	NH_4, M.Eq.	Increase Over Fore Period: Mineral Acid	Fixed Base	NH_4
1, 2, 3	.....	1070	0.52	4.4	20.4	37.0	32.0	89.4	52.1	18.7	....	....	....
4	109.0	1200	0.56	4.4	63.6	48.9	33.1	145.6	110.4	23.2	56.2	58.3	4.5
5	109.0	1280	0.57	4.4	89.2	52.0	35.8	177.0	127.7	35.8	87.6	75.6	17.1
6	109.0	1025	0.52	4.8	70.5	30.6	31.9	133.0	81.4	43.3	43.6	29.5	24.6
7	54.5	1030	0.55	4.8	84.3	30.6	36.4	151.3	77.2	76.5	61.9	25.1	37.8
8	.....	1115	0.55	5.0	60.2	40.3	33.8	134.3	66.2	59.1	44.9	14.1	40.4
9	.....	lost	....	...	....	....	....	.....	.....	....	....	....	....
10	.....	900	0.58	5.2	19.9	45.7	31.9	97.5	43.8	45.8	8.1	—8.3	27.1
11	.....	960	0.52	5.2	17.1	40.0	29.0	86.1	37.2	38.2	—3.3	—14.9	19.5

* Reported by Gamble et al (footnote 10, p. 379).

there was advanced impairment, was somewhat greater than in the case of either the normal control or the nephritic patient with edema.

In attempting to account for the differences observed between normal subjects and patients with different types of nephritis in regard to the quantitative relations of mineral acid and fixed base excreted, it is necessary, aside from renal factors, to consider the changes that take place in the tissues. It seems that a change in the direction of acidosis decreases the capacity of the tissues to bind base and also water; that there is a store of extracellular base, chiefly sodium, which is readily

parted with, and that intracellular base, chiefly potassium, is drained by more severe or prolonged acidosis.[29] In conditions of edema the tissues tend to become loaded with increased quantities of sodium. Consequently, as Gamble and his associates[10] pointed out, the patient with edema would be expected to throw off abnormally large quantities of fixed base with the administration of acid. In the ordinary types of chronic glomerular or arteriosclerotic nephritis in adults there is usually

TABLE 4.—*Case 67985. Slightly Impaired Renal Function, with No Edema. Influence of Ammonium Sulphate on Excretion of Acids and Bases in Urine*

Day	$(NH_4)_2SO_4$ Ingested, M.Eq.	Urine Volume, Cc.	Creatinine, Gm.	p_H	SO_4, M.Eq.	Cl, M.Eq.	PO_4, M.Eq.	Mineral Acid, M.Eq.	Na, M.Eq.	Fixed Base, M.Eq.	NH_4, M.Eq.	Increase Over Fore Period: Mineral Acid	Fixed Base	NH_4
1	0	980	1.20	5.3	48.7	31.5	29.9	110.1	49.2	100.0	16.8	....	....	....
2	0	1140	1.32	5.3	53.2	24.4	30.4	108.0	54.0	107.2	22.1	....	....	....
3	0	1260	1.35	5.4	41.0	38.0	30.2	109.2	52.7	103.2	17.6	....	....	....
4	96	1450	1.20	5.0	63.8	44.5	38.2	146.5	69.0	134.1	22.8	37.3	30.6	4.0
5	96	1430	1.39	4.8	87.4	51.3	33.9	172.6	61.5	138.7	30.9	63.4	35.2	12.1
6	96	1560	1.32	4.4	85.3	53.0	38.0	176.3	54.0	133.0	38.4	67.1	29.5	19.6
7	96	1710	1.40	4.4	91.6	61.4	38.1	191.1	57.2	142.9	47.3	115.3	39.4	28.5
8	96	1740	1.35	4.4	89.0	50.6	37.4	177.0	56.0	141.0	42.8	81.9	37.5	24.0
9	0	1610	1.32	4.6	72.8	51.3	29.5	153.6	49.6	110.2	45.6	44.4	6.7	26.8
10	0	1220	1.15	4.9	37.3	28.9	32.2	98.4	41.0	86.0	27.5	—10.8	—17.2	8.7

TABLE 5.—*Case 67156. Advanced Renal Impairment, with No Edema. Influence of Phosphoric Acid on Excretion of Acids and Bases in Urine*

Day	H_3PO_4 Ingested, M.Eq.	Urine Volume, Cc.	Creatinine, Gm.	p_H	SO_4, M.Eq.	Cl, M.Eq.	PO_4, M.Eq.	Mineral Acid, M.Eq.	Na, M.Eq.	Fixed Base, M.Eq.	NH_4, M.Eq.	Increase Over Fore Period: Mineral Acid	Fixed Base	NH_4
1	0	1720	1.23	5.8	31.2	78.5	29.4	139.1	66.7	139.5	8.8	....	....	....
2	0	1770	1.28	5.8	28.0	86.0	29.6	143.6	67.3	138.2	7.1	....	....	....
3	110	2150	1.37	5.8	30.7	93.5	55.3	179.5	76.1	166.0	13.4	38.1	27.2	5.4
4	110	2180	1.26	5.0	33.2	106.1	70.0	209.3	74.1	190.9	20.5	67.9	52.1	12.5
5	75	1700	1.05	4.6	37.5	98.0	67.6	203.1	65.7	174.4	18.5	61.7	35.6	10.5
6	0	1890	1.15	5.0	38.7	96.8	43.5	179.0	48.0	155.5	19.3	37.6	16.7	11.3
7	0	1600	1.12	5.0	34.8	85.3	35.8	160.1	50.6	132.8	17.1	18.7	4.0	5.1

no edema except with heart failure or some other complication, and on account of the acidosis that accompanies the renal impairment the supply of easily mobilized fixed base of extracellular fluid should be somewhat depleted. Separate analyses do show low figures for extra sodium excretion in the nephritic patient (tables 4 and 5) as compared with the normal subject (table 2). This fact may account in part for the relatively small quantity of extra fixed base excreted in the urine

29. Haldane, J. B. S.; Hill, R., and Luck, J. M.: Calcium Chloride Acidosis, J. Physiol. **57**:301 (June) 1923. Gamble et al. (footnote 10).

during the first days of acid administration.[30] However, a much more severe grade of acidosis develops in the patient with renal impairment than in the normal control on a given dosage of mineral acid; so after prolonged administration more intracellular fixed base is released from the tissues of the nephritic patient.

In looking for renal factors to account for excretion of abnormally large quantities of fixed base with ingestion of acid, attention is again directed to the question of a diuresis that seems to handicap in some way the mechanism for conservation of base. The tables show that there was a definite and prolonged increase in the volume of urine in nephritic patients on ingestion of acid. A diuresis of the magnitude observed would probably not be great enough appreciably to handicap

TABLE 6.—*Case 68497. Advanced Renal Impairment, with No Edema. Influence of Ammonium Sulphate on Excretion of Acids and Bases in Urine*

Day	$(NH_4)_2SO_4$ Ingested, M.Eq.	Urine Volume, Cc.	Creatinine, Gm.	p_H	SO_4, M.Eq.	Cl, M.Eq.	PO_4, M.Eq.	Mineral Acid, M.Eq.	Fixed Base, M.Eq.	NH_4, M.Eq.	Increase Over Fore Period: Mineral Acid	Fixed Base	NH_4
1	0	2560	1.08	5.0	41.5	130.0	31.8	203.3	174.7	8.1	....	....	....
2	0	2940	1.22	4.9	46.2	124.4	41.2	211.8	210.9	8.6	....	....	....
3	0	2950	1.23	5.0	45.7	138.4	40.0	224.1	220.2	9.3	....	....	....
4	96	2540?	0.90	4.9	44.7	120.6	29.6	194.9	175.4	8.9	?	?	?
5	96	3100	1.15	4.8	81.6	144.1	36.4	262.1	247.2	12.4	49.0	45.2	4.1
6	96	3310	1.29	4.8	102.5	157.2	40.8	300.5	305.7	13.9	87.4	83.7	5.3
7	96	3320	1.30	4.9	107.4	143.9	42.8	294.1	265.8	16.5	81.0	63.8	7.9
8	96	3390	1.31	4.9	101.2	142.8	40.8	284.8	239.0	19.2	71.7	37.0	10.6
9	0	2950	1.19	4.9	82.3	124.5	38.9	245.7	207.5	18.3	32.6	5.5	9.7
10	0	2980	1.14	4.9	63.8	126.1	37.8	227.7	205.0	12.5	14.6	3.0	3.9

the base-conserving mechanism in health, but superimposed on the prevailing compensatory diuresis in a kidney remnant, it might be expected tremendously to increase the rate of flow through the tubules and so lead to excretion of much extra fixed base. In addition to the pronounced increase in the excretion of fixed base, there is a conspicuous increase in excretion of chloride and a lesser increase in excretion of phosphate, results that are also consistent with a diuretic effect. In the experiment of Gamble (table 3) the drop in the excretion of fixed base was associated with a drop in the volume of urine.

An additional experiment has been conducted to bring out the changes in excretion of inorganic acids and bases with fluctuations in the volume of the urine caused by decreasing and increasing the intake of distilled

30. In one case of nephritis without edema studied by Linder (footnote 28) a surprisingly large quantity of potassium was excreted at the onset of acid administration. Some quantitative error was indicated, however, since the p_H values show that the urine was more acid, while extra fixed base excretion considerably exceeded the extra mineral acid excretion.

water. The results in table 7 show, with a diuresis of the order produced in the previous experiments, an increase in excretion of inorganic acids and a considerably greater increase in the excretion of fixed base. The changes in fixed base are not so great as those obtained by the administration of acid, presumably because the acid effect in the tissues is lacking.

The conclusion to be drawn is that changes in the excretion of fixed base caused by the administration of acid may be accounted for by a movement of fixed base from the tissues to the blood stream and to the urine and by a diuretic effect that handicaps reabsorption.

THE RÔLE OF THE AMMONIA SECRETED IN THE URINE

Since ammonia formation in nephritis is depressed along with functional impairment, considerable interest has necessarily centered on the relation of this activity to the inorganic disturbance. The strong impres-

TABLE 7.—*Case 68497. Advanced Renal Impairment. Influence of Distilled Water Diuresis on Excretion of Acids and Bases in Urine*

Day	Water Ingested, Cc.	Urine Volume, Cc.	Creatinine, Gm.	SO_4, M.Eq.	Cl, M.Eq.	PO_4, M.Eq.	Mineral Acid, M.Eq.	Fixed Base, M.Eq.	NH_4, M.Eq.	Increase Over Fore Period: Mineral Acid	Increase Over Fore Period: Fixed Base	Increase Over Fore Period: NH_4
1	2500	2620	1.20	38	102	41	181	152	18	....	....	...
2	1500	2040	1.20	35	77	38	150	118	21	−31	−34	+3
3	4000	3680	1.32	42	107	48	197	183	22	+16	+31	+4

sion gained during the course of this study is that the formation of ammonia serves chiefly to prevent excess acidity of the genito-urinary tract.

It has been observed in the first place that there is no serious depletion of serum fixed base even with advanced functional impairment, and the threshold values obtained may very well be due to a sustained compensatory diuresis. Then it was pointed out that the influence of acid on fixed base excretion in either normal conditions or different types of nephritis could be accounted for by a consideration of the effect of the acid on the base and water binding capacity of the tissues and the effect of the diuresis on reabsorption of fixed base.

The conventional teaching [31] that the formation of ammonia serves as an important factor in neutrality regulation in the body seems an unavoidable conclusion only as long as one supposes the ammonia to be formed in the tissues and neutralized by metabolic acids before reaching the kidney. The widely accepted view at present, however, is that the ammonia is formed by the kidneys,[32] although this was vigorously

31. Henderson, Lawrence J.: A Critical Study of the Process of Acid Excretion, J. Biol. Chem. **9**:403, 1911.

32. Benedict, Stanley R., and Nash, Thomas P.: On the Question of the Origin of Urinary Ammonia, J. Biol. Chem. **82**:673 (June) 1929.

opposed by Bliss.[33] The view that ammonia is formed to prevent excess acidity carries the implication that a primary secretion of high acidity is formed, since the bladder urine is slightly acid even after the formation of much ammonia. The interesting observations of Macallum and Campbell [34] indicate that a primary secretion of high acidity is formed. In this study of Macallum it was observed after intravenous administration of a solution of ferric ammonium citrate and sodium ferrocyanide to fasting experimental animals that prussian blue was formed in the cells of the proximal convoluted tubules and carried down into the urine. Prussian blue is not formed when the solution is added to a saturated solution of potassium dihydrogen phosphate, as was pointed out by Macallum. When it is added to tenth-molar lactic acid, a greenish coloration is formed, and in the presence of stronger acid the intense coloration of prussian blue is observed.

If, as Macallum believes, the primary renal secretion has an acidity comparable with that of gastric juice, it follows that the essential of fixed base conservation, i. e., formation of a secretion containing a large excess of acid over fixed base, is effected in the proximal convoluted tubules; that the ammonia is added later, or the primary secretion would not have been more acid than the bladder urine, and that the primary secretion, if not neutralized by ammonia or some other base, would be extremely irritating to the genito-urinary tract. It is obvious that whether the conventional view or the one suggested here is correct, ammonia and fixed base are complementary factors in neutralizing the acid of the urine, and with administration of alkali ammonia formation is depressed. In the curves showing the results of the administration of acid one observes about the same activity in the formation of ammonia on the second day of acid administration, a period of base waste, as on the second day of the after-period, a period of base conservation. In both cases the quantity of ammonia formed is just about sufficient to neutralize the excess of mineral acid over fixed base. In certain other cases the quantity of ammonia formed is not determined by waste or conservation of base but by the excess of acid over fixed base that is being excreted. One of these conditions is in the diuresis produced by drinking distilled water; increased quantities of fixed base are lost by the tissues into the urine, but the formation of ammonia is not stimulated. Another case is found in the study of Fiske and

33. Bliss, Sidney: The Amide Nitrogen of Blood: III. Muscular Exercise: The Role of Ammonia in the Neutralization of Lactic Acid, J. Biol. Chem. **81**:137 (Jan.) 1929.

34. Macallum, A. B., and Campbell, W. R.: The Secretion of "Acid" by the Kidney, Am. J. Physiol. **59**:439 (Oct.) 1929.

Sokhey.[35] They observed that when mineral acid was injected intravenously at a very slow rate, considerable extra fixed base was excreted and some extra ammonia formed, but when the acid was injected slightly faster, the respiratory center was so violently stimulated that alkalosis developed, more fixed base than before was lost from the tissues, and yet ammonia formation was depressed.

RELATION OF CERTAIN CLINICAL CONSIDERATIONS TO THE INORGANIC DISTURBANCE

Polyuria.—The secretion of large volumes of urine in chronic nephritis with retention of waste products has already been accounted for on the basis of a sustained compensatory diuresis in the kidney remnant. In order to obtain a clearer picture it may be supposed that, whereas in health about 150 liters of glomerular fluid are formed in a day and 1 per cent escapes reabsorption, leaving a volume of urine of 1.5 liters, with moderately advanced impairment only 50 liters of glomerular fluid are formed and 4 per cent escapes reabsorption, owing to the increased rate of flow, leaving 2 liters of urine.

Acidity of the Urine.—The urine tends to be more acid than normal with advanced renal impairment.[36] Compared with a normal subject on the same diet, a nephritic patient excretes similar quantities of the various acid waste products because of higher equilibrium levels in the blood stream. The level of fixed base in serum, and presumably that of glomerular fluid, is maintained at a slightly lower level with renal impairment, but the reabsorption of fixed base is not quite so perfect. The result is that the nephritic patient excretes about the same quantity of fixed base as the normal control on the same diet (Gamble et al.[10]). The greater acidity of the urine in nephritis depends, therefore, on the formation of less ammonia. The interpretation offered here is that the primary secretion of high acidity is not so completely neutralized by the formation of ammonia, because it is forced at a relatively high speed through relatively few tubules.

Edema.—There are two factors operating in nephritis with destruction of glomerular tissue that tend to prevent rather than to cause edema. The first is the poor tubular reabsorption and polyuria resulting

35. Fiske, Cyrus H., and Sokhey, S. S.: Ammonia and Fixed Base Excretion After the Administration of Acid by Various Paths, J. Biol. Chem. **63**:309 (March) 1925.

36. Henderson, Lawrence J., and Palmer, W. W.: On the Intensity of Urinary Acidity in Normal and Pathological Conditions, J. Biol. Chem. **13**:393, 1912-1913. McCorvie, John E.: Studies on the Morning Alkaline Tide of Urine in Normal Persons and in Patients with Nephritis, J. Clin. Investigation **2**:35 (Oct.) 1925. Magnus-Levy, A.: Das Ammoniak bei der Nephrose, Ztschr. f. klin. Med. **112**: 256, 1930.

apparently from an increased rate of flow. The other factor is the acidosis, which decreases the power of the tissues to bind water. Edema, when occurring in the arteriosclerotic types of nephritis, is nearly always due to heart failure, and the impression in this clinic is that patients with cardiac disease and renal impairment have less trouble with pulmonary and subcutaneous edema than those with good renal function. If there is any renal factor, aside from that which permits albuminuria, tending to cause edema, it should be looked for in the activity of the tubules as well as in that of the glomeruli, since the tubules are thought to be especially concerned with return of water and fixed base to the tissues. It is interesting to observe in this connection that the types of kidney disease associated with edema are those in which cells of the renal tubules show edema and various degenerative changes with little atrophy or necrosis, i. e., changes that might be looked on as irritation phenomena. If these changes are compatible with excessive functional activity,[37] then excessive quantities of water and fixed base would be returned to the tissues and would tend to cause scanty urine and edema. The levels of creatinine and other waste products in the blood stream and the quantities excreted in the urine are usually normal in the degenerative types of nephritis, in which edema is most troublesome. Calculations by the method of Rehberg,[24] therefore, indicate the formation of a normal quantity of glomerular fluid with scanty urine. The conclusion, then, seems forced that excessive reabsorption of water does occur as a cause of edema in degenerative nephritis.

Uremia.—The group of symptoms associated with the uremic syndrome are those to be expected from asphyxia, particularly asphyxia of various centers in the brain. In acute hemorrhagic nephritis, uremic manifestations are controlled by agents that relieve the edema of the brain (Blackfan[38]). When nephrosis terminates fatally, coma or other signs of uremia may be attributed to edema of the brain. In the arteriosclerotic type of nephritis, circulatory disturbances in the brain due to blood vessel changes or cardiac stasis are probably the most important factors in causing any manifestations of uremia that may be present.

The question of special interest here is whether or not the acidosis may be a contributing cause in the production of uremia. Koehler[39] found that administration of more than 15 Gm. of phosphoric acid or

37. Magnus-Levy (footnote 36, third reference) observed increased capacity of the kidney in nephrosis to produce ammonia and attributed this to an irritated state of the tubular epithelium.

38. Blackfan, Kenneth D.: Acute Nephritis in Children, with Special Reference to the Treatment of Uremia, Bull. Johns Hopkins Hosp. **39**:69, 1926.

39. Koehler, Alfred E.: The Effect of Acid and Base Ingestion upon the Acid-Base Balance, J. Biol. Chem. **72**:99 (March) 1927.

large doses of ammonium chloride or calcium chloride daily for a week or more to persons with normal kidney function gradually produced a marked fall in plasma carbon dioxide and p_H. With this severe prolonged acidosis he observed the following symptoms: headache, loss of appetite, nausea, drowsiness and muscle pains. These are the same symptoms found by Feinblatt [40] to be especially characteristic of uremia. Koehler pointed out that the symptoms are probably the result of tissue asphyxia, since tissue oxidation is at its optimum at a normal p_H. It would seem then that, although the chief causes of the symptoms of uremia that appear in the various types of nephritis are due to edema or circulatory disturbances in the brain, the presence of severe acidosis due to retention of phosphoric and sulphuric acids might increase the tendency toward asphyxia and be a contributory factor in producing the syndrome. In types in which edema of the brain is present, however, the analysis is complicated by the fact that the acidosis tends to reduce the edema. Acidosis up to a certain extent could then be beneficial when a greater degree would be detrimental.

That there is a decreased tolerance to mineral acids in chronic nephritis with impaired renal function is certain. This fact was observed by Gamble and his associates [10] and Linder [28] for sulphuric and hydrochloric acids. In the present study a number of patients with arteriosclerosis and impaired renal function showed poor tolerance to phosphoric acid or ammonium sulphate. In case 64392 the administration of phosphoric acid, 6 Gm. daily, was begun on Feb. 2, 1929, and on February 4, headache and nausea developed, symptoms that disappeared promptly when the phosphoric acid was discontinued. The patient in case 64838, with more advanced renal impairment, started to take phosphoric acid, 6 Gm. daily, on March 5, 1929, and generalized muscle pains and loss of appetite developed the following afternoon. The patient in case 67156, who had arteriosclerosis and advanced renal impairment and was given the same dosage of phosphoric acid, on the third day complained of general malaise, muscle tremors and nausea. The patient in case 67024, with advanced arteriosclerosis and renal impairment, took ammonium sulphate equivalent to 96 cc. of normal sulphuric acid, starting on March 13, 1930. He was observed to be quite drowsy and to vomit his evening meal on March 16. The patient in case 68497, with advanced renal impairment but not such extensive arteriosclerosis, was able, during the metabolism test starting Oct. 8, 1930, to take ammonium sulphate equivalent to 96 cc. of normal acid daily for five days without developing any symptoms of uremia, although blood taken on the last day showed a lower level

40. Feinblatt, H. M.: Uremia, the Syndrome of Nitrogen Retention, Boston M. & S. J. **189**:399 (Sept. 20) 1923.

of p_H than was observed with the onset of uremic symptoms in cases 67024 and 67392. The suggestion is that in the last patient, who had less advanced cerebral arteriosclerosis, there was less tendency to cerebral asphyxia. Other patients with rather advanced arteriosclerosis and only slight impairment of renal function have been able to tolerate considerably greater quantities of ammonium sulphate or phosphoric acid.

Treatment.—The problem in treatment for the acidosis in nephritis is to replace fixed base depletion and to promote excretion of, or limit accumulation of, sulphuric and phosphoric acids. The results of this study tend to minimize considerably the necessity for replacement of fixed base, since in uncomplicated cases the level of fixed base is scarcely below the limits of normal fluctuation.

The influence of alkali on the retention of acid in nephritis has been chiefly concerned with phosphoric acid. Marriott and Howland,[41] in an early study, found no evidence that administration of sodium alkali promoted excretion of phosphate in nephritis. In a previous study,[42] I observed that the administration of potassium or magnesium acetate failed to influence the excretion of phosphate in the urine. Administration of calcium salts, however, has been shown to decrease the excretion of phosphate in the urine [43] and to increase excretion in the feces, presumably because unabsorbed calcium tends to bind phosphoric acid split off during digestion, and calcium that is absorbed is excreted in the feces for the most part and carries phosphate along in combination. It will be observed that the patients in cases 62367 and 64838, who took calcium carbonate, 6 Gm. daily, did show a definite drop in the level of serum phosphate. Marriott and Hartmann [44] also spoke of reducing the level of serum phosphate in nephritis by calcium therapy.

Along this same line some attention has been given to the question of strontium therapy. Strontium, like calcium, is excreted chiefly in the feces and has an insoluble sulphate as well as a phosphate. It is conceivable, then, that strontium therapy in chronic nephritis might facilitate the removal through the intestine of sulphuric as well as phosphoric acid. Intraperitoneal administration of strontium acetate

41. Marriott, W. McKim; and Howland, J.: Phosphate Retention in Nephritis, Arch. Int. Med. **18**:708 (Nov.) 1916.

42. Briggs, A. P.: Some Metabolic Aspects of Calcium Therapy, Arch. Int. Med. **37**:440 (March) 1926.

43. Telfer, S. V.: Studies in Calcium and Phosphorus Metabolism: III. The Absorption of Calcium and Phosphorus and Their Fixation in the Skeleton, Quart. J. Med. **17**:245, 1924. Briggs (footnote 42).

44. Marriott, W. McKim; and Hartmann, Alexis F.: Newer Aspects of Acidosis, J. A. M. A. **91**:1675 (Dec. 1) 1928.

in dogs caused a conspicuous drop in the urinary excretion of phosphate and a very slight drop in sulphate, suggesting that strontium phosphate and sulphate had been excreted in the feces.[45] In the present study, a drop in the levels of both serum phosphate and sulphate after administration of strontium carbonate, 6 Gm. daily, was observed in the following instances: in case 64123, on Dec. 29, 1928; in case 66998, on April 21, 1930; in case 67392, on April 25, 1930, and in case 68497, on Dec. 20, 1930. With the drop in sulphate and phosphate there is a slight rise in bicarbonate and p_H, that is, a partial correction of the acidosis.

Having partially corrected the acidosis, it would be gratifying to be able to report associated clinical improvement. As pointed out, however, this work has been done on patients without symptoms. Moreover, the early appearance of nausea as a symptom has so far interfered with the use of strontium therapy in uremia. It will be interesting to see, however, if the continuous administration of calcium or, better, of strontium carbonate to a nephritic patient will prolong the course before the appearance of symptoms.

In attempting to evaluate the efficacy of the high base diets introduced by Sansum, Blatherwick and Smith [46] for treatment in nephritis, a number of points should be observed. Evidence was just presented that the sodium and potassium alkalis of such diets do not promote excretion of phosphoric acid, and in addition it might be mentioned that Shohl and Sato [47] found that administration of sodium bicarbonate failed to influence appreciably the excretion of any acid. Moreover, a diet consisting chiefly of fruits and vegetables has been found to provide insufficient protein in cases in which albuminuria is severe.[48] When protein (high phosphorus and sulphur) foods are incorporated in the diet, nothing seems to be accomplished in the way of eliminating the resulting acids by ingestion of a quantity of fruit and vegetables (sodium and potassium alkali). A special case is observed when milk is used to provide the necessary protein. In this instance the high phosphorus content of the milk is balanced by a high calcium content, which seems to facilitate the elimination of phosphorus through extrarenal channels. From the foregoing evidence it would seem that the ordinary high base diet has for its chief merit an associated limitation of phosphoric and sulphuric acids.

45. Unpublished experiments.

46. Sansum, W. D.; Blatherwick, N. R., and Smith, Florence H.: The Use of Basic Diets in the Treatment of Nephritis, J. A. M. A. **81**:883 (Sept. 15) 1923.

47. Shohl, A. T., and Sato, A.: Acid-Base Metabolism: II. Mineral Metabolism, J. Biol. Chem. **58**:257 (Nov.) 1923.

48. Peters, John P., and Bulger, Harold A.: The Relation of Albuminuria to Protein Requirement in Nephritis, Arch. Int. Med. **87**:153 (Feb.) 1926.

SUMMARY AND CONCLUSIONS

The acid-base equilibrium has been studied in a group of nephritic patients in whom vomiting and other complications were negligible.

It has been observed that the condition of the serum acids is very similar to that found after ligation of both ureters in experimental animals. There is, however, a very slight depression in the level of serum fixed base. It has been observed that prolonged administration of mineral acid to these nephritic patients (without edema) leads to waste of more base than in normal controls.

It has been concluded that the defect in conservation of base, as well as of water, chloride and other substances, depends largely on an increased rate of flow of the glomerular fluid through the surviving tubules.

It has been concluded that the chief function served by ammonia formation is the prevention of excess acidity in the genito-urinary tract.

An attempt has been made to apply the experimental findings and theoretical considerations to various clinical and therapeutic features of nephritis.

TUBERCULOSIS AMONG NURSES*

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The subject of this paper is not new, but it has not been given adequate attention, and because of its importance merits extensive study. Heimbeck[1] in Norway, Ross[2] in Canada, Shipman and Davis,[3] and Whitney[4] in this country have sensed the problem and have suggested corrective measures that are satisfactory only in part, because none of these writers has sought to prevent contagion, which in my experience is the essence of the question. Furthermore, as this inquiry progressed it appeared to me that this might be a study that would throw more light on the pathogenesis of tuberculosis.

The present investigation was undertaken because there seemed to be an unusually high incidence of tuberculosis among student nurses at the Ancker Hospital in St. Paul. This institution is the city and county hospital for St. Paul and Ramsey County. It has 975 beds, 215 of which are devoted to patients with tuberculosis and form the Ramsey county sanatorium. The nurses for the tuberculosis pavilion come from the nurses training school of the hospital and in the main are from rural communities. Their average age on entering training is from 18 to 20 years. The nurses devote approximately four months of the three years of training to the care of patients with tuberculosis and part of this training is given the first year.

On looking over the records in the training school office, it was found that 42 nurses from a total of 934, or 4.5 per cent, had broken down with tuberculous disease from 1920 to 1928, either during training or shortly afterward. For many years, all nurses entering training have been given physical examinations, and beginning in 1926, roentgenograms of the chest have been taken. Preventive inoculations against

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1. Heimbeck, Johannes: Immunity to Tuberculosis, Arch. Int. Med. **41**:336 (March) 1928.

2. Ross, E. L.: Canad. M. A. J. **22**:347, 1930.

3. Shipman, S. J., and Davis, E. A.: Am. J. Nursing **23**:8, 1928.

4. Whitney, J. S.: Am. J. Nursing **23**:8, 1928.

smallpox and diphtheria are also given, weights are recorded periodically, and physical examination repeated when it is considered necessary.

It was thought that routine skin tuberculin tests might throw some light on the problem of tuberculosis, so in September, 1928, I began to test the incoming class of probationers. All the tests have been given intradermally, and, with the exception of the first two groups, I have used tuberculin made from the H 37 strain of tubercle bacillus sent to me by Dr. Allen K. Krause. One-tenth milligram has been given at the first injection, and if the reaction has been negative the test has been repeated in forty-eight hours with a dosage of 1 mg. The scale for reading the reactions has been essentially that used by Opie and McPhedran.

Of the 32 young women entering training in September, 1928, 10 gave positive and 22 negative reactions. This result was not surprising, as it was comparable to the findings of Lees and Myers [5] in testing freshman at the University of Minnesota, a similar age group from practically the same community.

I was curious to know what reaction the remainder of the training school students would show, and was amazed to discover that of 147 nurses, 143 gave positive reactions and only 4 negative. In other words, nearly all of the nurses who had been in training for six months or more were infected, in contrast to an entering group only one third of whom were infected.

These findings provided food for thought. Was it true that in Minnesota only from 30 to 35 per cent of the young people reaching the twenties had acquired tuberculous infection? Slater's [6] work tends to prove this, and Lees and Myers' [5] observations substantiate it, as do those of Laird, Guyer and Bailey,[7] although as late as 1929, Rich and McCordock [8] spoke of the rare adults "who escape the protective infection of childhood." It is known that tuberculous infection may become so thoroughly overcome that the skin reaction becomes negative. Opie and McPhedran,[9] Krause,[10] Austrian [11] and others have noted this,

5. Lees, H. D., and Myers, J. A.: Am. Rev. Tuberc. **21**:532, 1930.

6. Slater, S. A.: Am. Rev. Tuberc. **10**:299, 1924.

7. Laird, A. T.: Guyer, L. G., and Bailey, W.: Am. Rev. Tuberc. **24**:207, 1926.

8. Rich, O. R., and McCordock, H. A.: Bull. Johns Hopkins Hosp. **44**:370, 1929.

9. Opie, E. L., and McPhedran, F. M.: Am. Rev. Tuberc. **24**:362, 1926.

10. Krause, A. K.: Am. Rev. Tuberc. **11**:303, 1925.

11. Austrian, C. R.: Tubercle **6**:29, 1924.

although it is uncommon. In my experience in following up the children who have left the Children's Preventorium of Ramsey County since 1915, only 10 of more than 400 who gave positive reactions later gave negative reactions. A percentage such as this would not materially affect the results noted in the incoming nurses. In fact, all the evidence points to the conclusions that the incidence of tuberculous infection in young adults of Minnesota is low.

My next thought was whether or not this low incidence of tuberculous infection should influence the selection of the nursing personel for our tuberculosis institutions. I think it advisable in all sanatoriums and hospitals to give all nurses on entering training physical and roentgen examinations and to make skin tests. An important question is whether or not nonreacting nurses should be permitted to care for consumptive patients. Should such nurses be kept out of sanatoriums and away from tuberculosis departments of general hospitals? The old dictum that sanatoriums are the safest places to be in so far as tuberculosis is concerned finds little support in this investigation. Heimbeck's[1] use of B C G for his nonreacting nurses is hardly warranted as yet; furthermore, it is the wrong approach. Institutions can and should be made safe from the standpoint of contagion, and then there will be less cause to worry about nurses or other employees. In the light of present knowledge concerning the immunology of tuberculosis and in view of the inadequate medical aseptic measures now used in most tuberculosis institutions, I am convinced that nonreacting nurses who come in contact with tuberculous patients are in a more precarious situation than the reacting nurses, because a large majority of the former have not had the protective infection of childhood. Some doubtlessly will argue that nonreacting nurses should be kept away from tuberculosis institutions, and others will contend that nurses who exhibit a highly allergic state should also be excluded from nursing tuberculous patients. These apparent paradoxical points of view will have little support if precautions are taken to protect nurses from frequent and massive doses of tubercle bacilli. There must be nurses, so it is necessary to make their working environment safe. This implies not only careful general supervision of working hours, adequate food and opportunities for rest and recreation, but connotes rigid contagious technic when the nurses are on duty with tuberculous patients.

Since September, 1928, I have been testing all incoming nurses at the Ancker Hospital and retesting, every six months, those who gave a negative reaction. An interesting observation has been that those originally showing a negative reaction have had, without exception, 3

or 4 plus reactions when they have become positive, which must be interpreted as showing recent infection. The results of this periodic retesting is shown in the accompanying chart.

It seemed worth while to test nurses in other hospitals in St. Paul to see what the incidence of tuberculous infection was in institutions without tuberculosis departments. Table 1 shows the results of these tests.

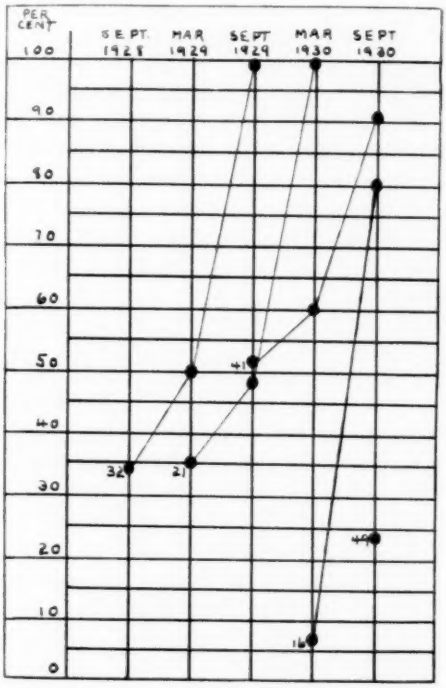

Chart showing the number of women entering training at Ancker Hospital, spring and fall, since September, 1928, the percentage of those who gave positive reactions originally and the rapid increase in this percentage at six-month intervals.

Likewise it appeared advisable to investigate a group similar to the Ancker nurses. The University of Minnesota Hospital Training School offered this opportunity, as its nurses are sent to Glen Lake Sanatorium for a period of from six to eight weeks. The results of the test are shown in table 2.

There is another group of nurses in the Ancker Training School that I have tested concerning which it is difficult to draw conclusions. Since September, 1928, I have tested 114 nurses who have been affiliated

with the Ancker School or transferred from other hospitals. In the main, these nurses have come from small institutions in the Northwest, and the time they have spent in training elsewhere has varied. Of these 114, 81 gave a positive reaction, and 33, a negative one, a percentage incidence of those giving positive reactions of 71 plus. It is interesting to compare this group with the group from other St. Paul hospitals.

Since 1928, Dr. F. F. Callahan has been testing nurses at Pokegama Sanatorium. This is a small private institution having a two

TABLE 1.—*Showing a Combined Tabulation of Tests of Student Nurses at Bethesda, Midway, Mounds Park, St. John's and St. Luke's Hospitals in St. Paul* *

Year in Training	Number Tested	Positive		Negative	
		Number	Per Cent	Number	Per Cent
First	95	38	40	57	60
Second	79	34	43.1	45	56.9
Third	45	19	42.3	26	57.7
Total	219	91	41.8	128	58.2

* The procedure used at Ancker Hospital of testing nurses on entering training and retesting every six months was impossible in these hospitals. By tabulating according to the year in training, the intention was to ascertain whether or not the incidence of infection increased with the length of time in training.

TABLE 2.—*Showing Results of Testing Student Nurses from the University of Minnesota Hospital Training School* *

Glen Lake Service	Number Tested	Positive		Negative	
		Number	Per Cent	Number	Per Cent
Before	291	138	47.4	153	52.6
After	65	55	84.6	10	15.4

* No attempt was made to separate this group into years of training. The purpose was to ascertain the difference, if any, between nurses who had had, and those who had not had, service at Glen Lake Sanatorium.

year training course which draws largely former patients as students. Consequently, the findings in this small group would necessarily vary from those in the Ancker group. In the past two years, 13 young women have entered training at Pokegama. Only 2 of them gave a negative reaction on admission, but the reactions became positive within a year. In one of those with a positive reaction pleurisy with effusion developed while she was in training, and in another a frank parenchymatous lesion developed; the latter nurse had a sister with pulmonary tuberculosis and a year before entering training this nurse had had a definite hemoptysis without other symptoms.

It appears incontrovertible that women entering the nurses' training school at Ancker Hospital have an average incidence of tuberculous infection of about 30 per cent, and that by the end of the three year

training period, and usually within the first year, practically all are infected, which is in agreement with Heimbeck's [1] findings. This is in startling contrast to what happens in other hospitals in St. Paul; their nurses show an incidence of 42 per cent in the last year of training. As all come from a similar age group in the same community, it seems fair to assume that the nurses from the Ancker Hospital acquire infection because part of their training brings them in contact with cases of open pulmonary tuberculosis.

As to the technic used in the tuberculosis pavilion of Ancker Hospital and as to whether or not patients receive instructions, all nurses going to the pavilion are told that they are handling a communicable disease. They are instructed and told repeatedly to wash their hands after giving bedside care and before going to meals. They are told to insist on patients covering the mouth and nose when coughing or sneezing. They are cautioned to keep their hands away from their nose and mouth. They wear gowns and gauze masks. They receive instruction as individuals and in small groups, as well as in formal class lectures. The patients are given monthly talks by radio covering the various phases of tuberculosis, besides innumerable individual and ward talks, and they are also encouraged to read books written for patients.

Despite these measures, the nurses become infected and some of them massively so. One serious obstacle to be overcome is a psychologic one. It is readily appreciated how a nurse in the contagious department, caring for a patient with diphtheria or scarlet fever, would be impressed by the acute illness and be meticulous in her personal precautions. It is just as understandable how she might unconsciously become careless in a ward of consumptives, where the appearance of some of the patients is such that the uninformed person wonders why they are kept in bed.

In order partially to overcome this difficulty and because, after all, tuberculosis is a communicable disease, may it not be wise to insist on the most rigid contagious technic in sanatoriums and tuberculosis departments of general hospitals, especially in those communities in which the incidence of tuberculous infection in young adults is low? It would impress nurse and patient alike, and it is my conviction that it should be done.

Another factor of importance, particularly in large municipal hospitals and probably to a less extent in private general hospitals, is the unrecognized, undiagnosed case of consumption. No nurse can protect herself fully against an infectious disease unless she knows what type of patient she is nursing. The number of persons sent into general hospitals for observation is large. Many elderly people are

admitted for a variety of complaints who give a history of an apparently innocuous bronchitis of many years' duration. A goodly number of persons in the teens are sent to hospitals for conditions that appear to be pneumonia, influenza or acute bronchitis, but after one, three or six weeks, it is found that tuberculosis is present. This unseen danger, the patient with an undiagnosed case of consumption, is a very real one to nurses, and I know from experience that it exists in the general wards at Ancker Hospital.

The solution of this difficulty lies to no small degree in: first, taking a roentgenogram of the chest of every patient admitted to general hospitals, just as urinalysis, hemoglobin and white cell counts are made so generally; second, instructing nurses to send to the laboratory all sputum that the patients may raise. These two measures should be incorporated into the routine of every hospital, as they would largely eliminate the menace of unrecognized consumption. At Ancker Hospital these measures, along with contagious technic in the tuberculosis department, are to be established for a trial period of at least two years, during which I shall continue the tests and examinations described previously to note any change in the incidence of tuberculous infection and disease. An attempt is also being made to reduce the time each nurse spends in the tuberculosis department.

The roentgenograms of the chests of the Ancker group were studied and compared with the results of cutaneous tests. This inquiry was not wholly satisfactory, because in a film taken with the usual technic root shadows are notoriously variable, and the presence of calcification is not always certain. In two instances calcified foci in the parenchyma were noted in nurses giving a 3 plus. Ten nurses showed diaphragmatic adhesions; five of the nurses gave positive reactions and five did not. Two parenchymatous infiltrations were noted, one positive and one negative which later became positive (case 4). The nurse with the parenchymatous lesion and positive skin test was permitted to continue working, as she was free from symptoms; the lesion has faded gradually to the point of being barely perceptible. I have begun to make repeated roentgen examinations of nurses who originally gave negative reactions as their reactions become positive, to see whether a childhood type of lesion develops. This investigation will be reported on later; so far it has revealed little, if anything.

The incidence of tuberculous disease among nurses has not been clearly established. In 1925 Britton and Bollman[12] stated that 2.2 per cent of all nurses employed in Chicago had tuberculosis. Heimbeck[1] found that 12 per cent of student nurses over a period of four

12. Britton, J. A., and Bollman, E. B.: Tr. Nat. A. Prev. Tuberc., 1925.

years developed the disease. Shipman and Davis [3] found that 2.6 per cent of nurses in the University of California Training School developed tuberculosis over a period of six years. In the Eppendorf Hospital, Much [13] found that the percentage incidence up to 1918 was 1, and that after the war it rose to 4.6. Ross [2] found that 6 per cent of 800 nurses in the province of Manitoba had developed tuberculous disease over a four year period. Against these figures there is the statement of Whitney [4] "that in the general population even with the very high death rate among young women from tuberculosis only about 1.5 per cent of them may be expected to develop tuberculosis."

What has happened to the 110 nurses who have entered training in the last two years at Ancker Hospital aside from their acquiring tuberculous infection? In 6, or 5.5 per cent, tuberculous disease has developed. This incidence is in contrast to the fact that in the other hospitals in St. Paul in which I have made tests no nurses have developed tuberculosis. Of the 6 nurses who had tuberculosis, 3 had pleural effusions, 2 parenchymatous lesions of the lungs of the adult type and 1 a lesion of the lower lobe, apparently of the childhood type. Five of these 6 nurses gave negative cutaneous reactions to 1 mg. of old tuberculin when they entered training. Abstracts of their histories are interesting.

REPORT OF CASES

CASE 1.—M. F., aged 20, entered training in February, 1928. There was no tuberculosis in the family nor any known contact. The patient gave a negative reaction to 1 mg. of old tuberculin injected intradermally in September, 1928. Physical examination and roentgenograms of the chest were negative.

The past history was unimportant. In January, 1929, the patient began to have pain in the right side of the chest, and shortly afterward she became short of breath on exertion and noticed fever. She was admitted to the hospital as a patient acutely ill, and effusion in the base of the right lung was found. The cutaneous reaction to 0.1 mg. of old tuberculin was 3 plus at that time. Diagnostic thoracentesis revealed serous fluid; no organisms were found. Lymphocytes predominated on the smear. Inoculation of a guinea-pig gave negative results.

CASE 2.—M. O'D., aged 21, entered training in September, 1928. There was no tuberculosis in the family nor any known contact. The patient gave a negative reaction to 1 mg. of old tuberculin; a physical examination and a roentgenogram of the chest gave negative results.

The past history was unimportant. In January, 1929, the patient began to have pain in the left side of the chest. She continued her work until May, 1929, when she had pain in the right side of the chest with unproductive cough. She was admitted to the hospital as a patient acutely ill, and pleural effusion of the right lung was found. On admission the cutaneous reaction to 0.1 mg. of old tuberculin was 3 plus. The pleural fluid was serous and contained no organisms; lymphocytes predominated on the smear. Inoculation of a guinea-pig gave negative results.

13. Much, H.: Beitr. z. Klin. d. Tuberk. **64**:155, 1926.

CASE 3.—E. C., aged 20, entered training in September, 1928. There was no tuberculosis in the family nor any known contact. The reaction to 1 mg. of old tuberculin was negative; physical and roentgen examinations gave negative results.

The past history was unimportant. In December, 1929, the patient began to lose weight. She had frequent colds. In May, 1930, she had pain in the chest. She reported for examination in July, 1930, when a definite parenchymatous lesion was found in the upper lobe of the right lung with a 3 plus reaction to 0.1 mg. of old tuberculin.

CASE 4.—G. S., aged 21, entered training in September, 1929. Her mother died of pulmonary tuberculosis in 1924. One maternal uncle had pulmonary tuberculosis. There was no reaction to 1 mg. of old tuberculin. The results of the physical examination were negative, but the x-ray films showed a small parenchymatous lesion in the apex of the right lung.

The past history was unimportant. The patient was admitted to the hospital as a patient in January, 1930, because of fatigue, loss of weight and fever in the afternoon. Roentgenologic indications of tuberculosis increased; the patient gave a 3 plus reaction to 0.1 mg. of old tuberculin.

CASE 5.—A. E., aged 20, entered training in 1929. There was no tuberculosis in the family nor any known contact. The reaction to 1 mg. of old tuberculin was negative; the results of physical examination and roentgenograms of the chest were negative.

The past history was unimportant. In April, 1930, the patient began to tire easily, and found that she had a temperature ranging from 99 to 100 F. in the afternoon; at no time was she acutely ill. She had a cough, with mucoid expectoration. There were physical signs at the base of the right lung, dulness, breath sounds suppressed and pleural friction. X-ray films showed a diffuse, dense, homogeneous shadow at the lower lobe of the right lung. Thoracentesis did not reveal fluid. The patient showed a 3 plus reaction to 0.1 mg. of old tuberculin.

CASE 6.—F. B., aged 20, entered training in September, 1928. Her maternal uncle had tuberculosis and visited in the home of the patient when she was from 8 to 12 years old. No record of a tuberculin test was made in September, 1928, but in October, 1929, the reaction was positive.

The past history was unimportant. The present illness began about June 5, 1930, when the patient experienced sharp pain in the chest. On July 13 she was admitted as a patient acutely ill, with a temperature in the afternoon as high as 103 F.; the cough was unproductive. There were signs of pleural effusion in the left side of the chest. Diagnostic thoracentesis revealed serous fluid which contained no organisms; lymphocytes predominated on the smear. Inoculation of a guinea-pig gave negative results.

In all of the cases reported, the nurses have recovered or are doing so, and at this time it seems that they will be able to complete their training.

Again one notes the substantiation of Heimbeck's [1] report to a large degree. Obviously, at least, as shown thus far, it is in the main those who originally gave negative reactions in whom tuberculous disease develops, in some instances within a very short time after exposure. Certainly, in adults it does not always require many months for tuberculous disease to develop, and it may develop soon after primary infection. Gor-

don and Cashman,[14] Norris and Landis,[15] Ross,[2] and others have expressed the belief that tuberculosis does not develop among nurses, doctors and other employees who are working in tuberculosis institutions, but this is happening to nurses at Ancker Hospital, and it is stretching one's credulity too far to assume that in this respect Ancker Hospital is unique among American institutions.

It is interesting to speculate why the six women became ill. They are from a group which, for its age, is as well controlled as possible, and which has a very uniform environment for over three years. Two came from families with tuberculous histories (cases 4 and 6). All were apparently healthy and robust without serious past illnesses. They worked the same number of hours in an identical occupation, ate the same kind of food and slept in similar quarters as the nurses who did not become ill. Krause and Willis[16] have stated that "under certain circumstances frequently repeated specific reinfection does depress both allergy and immunity" and that "specific intoxication is a potent factor in unfavorably influencing immunity." They also believe, "there is a dosage of reinfection and a spacing of intervals between reinfections one of which is always correlated with the other which are favorable for the highest development and increase of allergy and immunity—but there is also a dosage and a spacing which will depress and (perhaps) destroy each." Is it not probable that the latter type of treatment has been given to the nurses of the Ancker Hospital who have become ill? Is it also not probable that if these women had had an earlier mild infection they would not have become ill? Furthermore, is it not most likely that had these nurses had the protecting advantage of strict medical asepsis, they would have been subjected to fewer and lighter doses of bacilli, thereby escaping tuberculous disease? Of course, factually, one finds oneself confronted with two totally unknown and perhaps unknowable factors: the bacillary dosage that these nurses received and something called individual resistance, which must vary somewhat in each one of us. It is within our power now to control the first factor; perhaps some day in the future we shall be able to define, estimate and enhance the second.

CONCLUSIONS

1. Thirty per cent of the nurses entering Ancker Hospital Training School show positive reactions to the intradermal tuberculin test.

2. Practically all nurses at Ancker Hospital show a positive cutaneous reaction to tuberculin before completing training.

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14. Gordon, B., and Cashman, W. M.: Tuberculosis in Workers After Residence in a Tuberculosis Hospital, J. A. M. A. **94**:1643 (May 24) 1930.

15. Norris, G. W., and Landis, H. R. M.: Diseases of the Chest, ed. 3, Philadelphia, W. B. Saunders Company, 1929, p. 335.

16. Krause, A. K., and Willis, H. S.: Am. Rev. Tuberc. **14**:36, 1926.

3. Nurses in five other general hospitals in St. Paul reveal an average of 42 per cent reacting positively to tuberculin given intradermally in their last year of training.

4. In 6 of 110 nurses, or 5.5 per cent, entering Ancker Hospital in the past two years tuberculous disease has developed; five of them gave negative reactions on entering training and three of them had pleurisy with effusion.

5. Tuberculosis sanatoriums and tuberculosis departments of general hospitals should employ rigid contagious technic, especially in those communities in which the incidence of tuberculous infection among young adults is low.

6. All patients entering all hospitals should have a roentgenogram of the chest made as a routine procedure.

7. All sputums should be sent to the laboratory by nurses.

8. It is hoped that studies similar to this one, with the inclusion of medical students, interns and other hospital employees, will be undertaken in other institutions.

Drs. Elizabeth A. Leggett and E. G. Hubin, Fellows at Pokegama Sanatorium, and Dr. F. M. Feldman rendered assistance during some of the testing.

THE ABSORPTION OF GAS FROM ANY CLOSED SPACE WITHIN THE BODY

PARTICULARLY IN THE PRODUCTION OF ATELECTASIS AND AFTER PNEUMOTHORAX *

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The absorption, transportation and elimination of gases in the living body have long been subjects of theoretical interest and extensive investigation. They have now become matters of great clinical importance also. This is especially the case in regard to two conditions which appear at first sight to be diametrically opposed to each other in all essential characters. They are the collapse, or atelectasis, of a lung when a bronchus is closed, and the reexpansion of a lung that has been collapsed by pneumothorax. The forces involved can both inflate and deflate a lung.

It is therefore timely to set forth the mechanics of gas absorption from a lung or from any cavity in the body in which air or any other gas happens to be occluded. In the development of atelectasis the first stage is the complete shutting off and cessation of ventilation in a part or the whole of a lung.[1] The air then gradually disappears and produces collapse of the part. In this absorption of gas there is nothing peculiar to the lungs. The same process of absorption acts throughout the entire body, for air, or any other gas, introduced into any cavity or tissue of the body gradually disappears. The same forces that produce atelectasis when a bronchus is blocked induce also the absorption of air or any other gas in a pleural space after a therapeutic pneumothorax. As the gas is absorbed from the pleural space the collapsed lung is drawn outward and thus reinflated. In the one case an occluded lung is collapsed; in the other a collapsed lung is reexpanded. But the forces of absorption of gas are the same in both cases.

GAS PRESSURES IN LUNGS AND BLOOD

Knowledge of the pressures and movements of the respiratory gases of the blood has been won by the arduous labors of many investigators:

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* From the Laboratory of Applied Physiology and the Sloane Physics Laboratory, Yale University.

1. For references to the literature, see Henderson, Yandell: Acapnia as a Factor in Postoperative Shock, Atelectasis and Pneumonia, J. A. M. A. **95**:572 (Aug. 23) 1930.

Pflüger, Bohr, Haldane, Barcroft, Hasselbalch, Haggard and Henderson, Van Slyke and others, and is one of the most highly developed fields of physiology.[2] In the main these investigations have traced the behavior of the gases in normal respiration, but the knowledge won may be applied to abnormal processes as well. The main points involved are as follows:

In normal air at sea level there are approximately pressures of 160 mm. of oxygen, 600 mm. of nitrogen and 0.3 mm. of carbon dioxide. Each of these components is termed the partial pressure of the particular gas. Their sum total, plus a variable amount of water vapor, is the barometric pressure, which at sea level varies around 760 mm.

During normal breathing there are in the alveolar air of the lungs (partial) pressures of about 100 mm. of oxygen, 40 mm. of carbon dioxide, and 570 mm. of nitrogen, plus 50 mm. of water vapor. As air passes freely in and out of the lungs, the total gas pressure in the alveoli is necessarily that of the outside air: 760 mm. The blood flows from the lungs to the left side of the heart and on into the arterial system containing oxygen, nitrogen and carbon dioxide at almost exactly the same pressures that these gases have in the lungs.

In the tissues the blood loses oxygen and takes up a somewhat smaller amount of carbon dioxide, depending on the respiratory quotient at the time. But as the blood holds these two gases by two distinct mechanisms—oxygen combined with hemoglobin and carbon dioxide largely as sodium bicarbonate—the pressure of oxygen is lowered much more than that of carbon dioxide is raised. Indeed, owing to the interaction of these gases in the blood (the Bohr-Haldane reciprocal effects), a considerable amount of carbon dioxide may be taken up with little or no increase of its pressure, if there is simultaneously a considerable decrease of the oxygen content of the blood.

For these reasons the venous blood returns to the lungs with its oxygen at the approximate pressure of only 40 mm.: a lowering of 60 mm. below the pressure of oxygen in the arterial blood. The pressure of carbon dioxide is simultaneously increased, but to a much less extent, rising only 6 mm. or less. It is 40 mm. in the arterial blood and 46 mm. or less in the venous. The pressure of nitrogen is unchanged, 570 mm. in both arterial and venous blood. The pressure of water vapor is also unaltered at 50 mm., as it is determined solely by the temperature.

2. For reviews of the literature, see Haldane, J. S.: Respiration, New Haven, Conn., Yale University Press, 1922. Henderson, Y., and Haggard, H. W.: Noxious Gases and the Principles of Respiration Influencing Their Action, American Chemical Society Monograph Series, New York, Chemical Catalog Company, 1927. Campbell, J. A.: Gas Tensions in the Tissues, Physiol. Rev. **11**:1 (Jan.) 1931.

The total of the pressures (40+46+570+50 mm.) for venous blood is thus only 706 mm., while the total alike for the arterial blood, the alveolar air and the air pressing on the body from without is 760 mm. This decrease of total gas pressure of about 54 mm. in the venous blood, due to the exchange of oxygen and carbon dioxide, plays an essential part in the normal process of respiration. It is also the factor which prevents the development or maintenance, except temporarily, of any gas-filled space in the body. This difference of gas pressure induces the absorption of gases from the pleural cavity in pneumothorax. Unfortunately it is also a factor of critical importance in the production of atelectasis in any portion of the lung which for a time is not ventilated.

As the total gas pressure in the venous blood is always less than that of the outside air, it is impossible for occluded gas in any part of the body ever to come into such equilibrium with the venous blood that it continues unabsorbed. To be in equilibrium the total pressure of the occluded gases would have to be only 706 mm. But the pressure of the outside air acting through the soft tissues of the body compresses any gas-filled space until its pressure is 760 mm. The total gas pressure in the space is then about 54 mm. higher than the total gas pressure in the venous blood flowing through the tissues surrounding it. This difference of gas pressure leads to absorption, more or less rapid, depending on the solubility and diffusibility of the various gases.

THE PROCESS OF GAS ABSORPTION

The details of the process of absorption in the development of atelectasis are, however, somewhat more complicated than this general explanation would indicate. As three gases are involved with different degrees of solubility and rates of diffusion through moist membranes, the process of absorption occurs in two distinct stages. During the first stage, immediately after a bronchus is occluded, the oxygen in the alveolar air at a pressure of 100 mm. diffuses into the (venous) blood in which the oxygen pressure is only about 40 mm. Simultaneously, carbon dioxide diffuses from the blood into the alveolar air, but in less amount, since the difference of pressure of 6 mm. in the blood and in the air is quickly equalized. Only a few seconds are required for the occluded air in this way to lose oxygen to the blood from a pressure of 100 mm. to 40 mm., and for carbon dioxide to increase in the air from 40 mm. to 46 mm. If the lung were a rigid box there would thus be induced a decrease of total pressure of 54 mm. But, as the lung has a structure which yields readily to the slightest change of pressure, what actually occurs is a decrease of volume.

There is now less gas in the occluded lung than initially and its volume is decreased. But the total gas pressure within it is nearly the same as before. The pressure of the air in the normal lung is 760 mm., and the pressure on the exterior of the body, which is transmitted through the abdominal viscera on the diaphragm, is also 760 mm. The occluded lung therefore shrinks, or is compressed by the pressure in the normal lung and that on the abdomen, until the pressure within the occluded lung is also 760 mm., minus a small force due to the elasticity of the other lung. The first decrease of volume in the occluded space is now complete and a second stage then sets in.

ABSORPTION OF NITROGEN

As nitrogen is much less soluble and therefore diffuses through the membranes of the lung more slowly than the other gases, the amount of nitrogen in the occluded air is at first little changed. But, as the volume of the occluded lung has decreased, the unchanged amount of nitrogen in it is compressed into a smaller space. For this reason the partial pressure of the nitrogen is increased.

The pressure of nitrogen is now 624 mm., which is 54 mm. higher than it was originally and 54 mm. higher, therefore, than the pressure of nitrogen in the venous blood. It is higher, in fact, by exactly the amount that the sum of the partial pressures of oxygen and carbon dioxide has fallen. The pressures in the occluded lung are then: oxygen 40 mm., carbon dioxide 46 mm. and nitrogen 624 mm., plus water vapor 50 mm. (total 760 mm.), while the pressure of nitrogen in the blood is still 570 mm. Under this difference in the pressure of nitrogen in the occluded air and that in the blood, this gas gradually diffuses from air to blood and is carried away by the circulation. As this occurs the pressures of oxygen and carbon dioxide are left a little above those in the venous blood, and these gases also pass from air to blood, for the total pressure in the occluded air is always nearly 760 mm. Meanwhile, owing to the continued absorption of the oxygen from the blood in the tissues, the pressure continues to be only 706 mm. in the venous blood flowing through the occluded lung.

This process continues until no gases are left; for the oxygen and carbon dioxide are always slightly, and the nitrogen considerably, above the pressure of each in the venous blood. Meanwhile the barometric pressure of the atmosphere, acting through the open parts of the lung and on the abdomen, presses the mediastinum over and the diaphragm up, until complete collapse of the finally apneumatic lung or lobe results. The alveolar surface and the blood flow are both large, and the absorption is therefore rapid.

If, instead of the air being occluded in a lung, the condition is that of a therapeutic pneumothorax, the air in the pleural space is gradually

absorbed into the venous blood. The collapsed lung is reexpanded simultaneously by atmospheric pressure, provided that the outside air can enter its airways and alveoli freely. But the surface and blood flow in the pleura are less than within the lung, and the absorption is therefore slower.

If, instead of a sea level barometric pressure of 760 mm., the prevailing barometer is considerably lower, as it is in mountain altitudes, the process of gas absorption occurs in essentially the same manner as at sea level. Indeed the oxygen pressure of the venous blood tends to fall even further below that of the arterial blood than it does when the oxygen in the air breathed is at its sea level value. Through the interaction of oxygen and carbon dioxide in the blood the pressure of carbon dioxide is also reduced. The absorption of gas from any occluded space is thus accelerated. This fact may explain why pneumonia commonly assumes a fulminating character in elevated localities.

On mechanical grounds no other mechanism than that actuated by these gas pressures is needed to account fully for the process of gas absorption in an occluded lung or in a pleural space. In the absorption the hydrostatic pressure of the blood in arteries, veins and capillaries, the so-called blood pressure, plays no part whatever. Blood pressures are the means by which the flow of blood around the circulation is induced, but they are not factors in the diffusion of gases.

Not only does the foregoing description fit the requirements of theory, but it is also in accord with a wide range of physiologic and clinical observations by many investigators. In particular it is in accord with the recent observations of Coryllos and Birnbaum[3] on the conditions in an occluded lung during the development of atelectasis.

CONCLUSION

The development of atelectasis is explainable on the basis of well established principles of physiology. The forces involved are essentially the same as those that control the diffusion of gases in normal respiration. The same principles and forces account also for the absorption of air from a pleural space after a therapeutic pneumothorax, and indeed for the absorption of gas from any space in the body.

The pressure of oxygen in venous blood is about 60 mm. lower than in arterial blood. The pressure of carbon dioxide is only about 6 mm. higher in venous blood than in arterial blood. On this account,

3. Coryllos, P. N., and Birnbaum, G. L.: Alveolar Gas Exchanges and Atelectasis: The Mechanism of Gas Absorption in Bronchial Obstruction, Arch. Surg. **21**:1214 (Dec.) 1930.

the total gas pressure in venous blood is always considerably less (60 — 6 = 54 mm.) than the total gas pressure of the atmosphere on the exterior of the body. The pressure of air in the lungs and of the atmosphere on the outside of the body compresses any gas-filled cavity, such as an occluded lung or pleural space, until the total gas pressure within it is nearly the same as that of the atmosphere. This pressure is therefore higher than the total gas pressure in the venous blood. Each gas then diffuses into the blood at a rate dependent on its own partial pressure, its solubility and the capacity of the blood for it.

As rapidly as gas is absorbed, the cavity is compressed by the pressure of the atmosphere on the soft tissues. As oxygen and carbon dioxide are absorbed quite readily, the partial pressure of nitrogen in the occluded air is thus raised above the pressure of this gas in the blood, and it gradually diffuses and is carried away by the blood. The rapidity of absorption is determined by the vascularity and surface area of the part.

In the absorption of gases, blood pressure is not at all involved. The only forces acting are the pressures of the gases in the occluded space and in the blood, and the atmospheric pressure in the normal parts of the lungs and on the exterior of the body.

EXPERIMENTAL AGRANULOCYTOSIS

INFECTION OF RABBITS WITH SALMONELLA SUIPESTIFER BY WAY OF THE BLOOD STREAM *

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Leukopenia accompanies numerous infectious and toxic conditions. However, the diminution in the number of the white corpuscles is oftentimes only transitory, and the drop in the leukocyte count, although it may have considerable prognostic significance, is not regarded as anything more than a symptomatic indication of a variety of disorders.

Recently, however, a condition has been described in which the outstanding characteristic, persisting until death of the patient, is the disappearance from the circulating blood of the polymorphonuclear leukocytes (granulocytes), the erythropoietic and lymphopoietic systems being but slightly affected. The clinical picture of the disease, as described by various authors, resembles that of a severe infection with fever accompanied by necrotic changes in various mucous membranes, particularly those of the buccopharynx. The patients, in the majority of instances women, not uncommonly develop jaundice. The disease is fatal in about 90 per cent of the cases. Blood cultures during life or post mortem are either sterile or yield various micro-organisms such as streptococci, B. coli or B. pyocyaneus.

Since Schultz'[1] original description in 1922, the disease has been designated as agranulocytic angina, agranulocytosis, malignant neutropenia or aplastic leukopenia or neutropenia. As stated, pathologic and hematologic investigations, performed on human material, have failed to disclose the etiologic factor of the malady.

In the experiments to be reported, an attempt was made to reproduce in animals a septic disease and to study the possible relationship between the hematopoietic system as seen in agranulocytosis in man and that of this form of sepsis found in the animal.

MATERIAL AND TECHNIC

Full grown rabbits weighing from 1.5 to 2 Kg. were used in the experiments, the animals being fed on oats, hay and small amounts of green vegetables.

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* From the Medical and Pathological Departments of the Beth Israel Hospital.

1. Schultz, Werner: Ueber eigenartige Halserkrankungen: Gangränes zierende Prozesse und Defekt des Granulosystems, Deutsche med. Wchnschr. **48**:1495, 1922.

Salmonella suipestifer, secured from the American Type Culture Collection, was chosen, first because this micro-organism has been described by some workers [2] as having a special affinity for the myelopoietic system, leading to a neutropenia of the infected animals; second, because of our intention of inaugurating a study of the hematologic findings in experimental sepsis by the use of a micro-organism that is essentially pathogenic for animals.

The salmonella was grown on plain agar slants, and a twenty-four hour old culture uniformly suspended in physiologic solution of sodium chloride was used in the experiments.

The number of bacteria in the suspension was counted before each experiment, and 1 cc. of the solution was injected into the marginal vein of the rabbit's ear. Leukocyte counts and blood smears from the peripheral blood were made at different intervals after the hematogenous infection. The dry films were studied with Wright's stain, preparations also being made with the supravital technic of Sabin. The bone marrow and other tissues were fixed and stained with the customary methods. The animals were killed by air embolus at different intervals after the infection, ranging from five minutes to several days.

EXPERIMENTS

The experiments may roughly be divided into two groups according to the number of bacteria injected: (1) relatively small doses, i. e., between 200,000,000 and 500,000,000 bacteria, and (2) large doses, i. e., between 2,000,000,000 and 5,000,000,000 micro-organisms.

The following is a résumé of protocols illustrative of the entire series of experiments.

Group I (relatively small doses).—*Rabbit 169* (fig. 1).—This animal, infected with 200,000,000 organisms, died in four days. Slight leukopenia due to decreases in granulocytes and lymphocytes developed within a few minutes. Immature polymorphonuclear leukocytes began to increase within an hour and reached their peak in three hours and forty minutes, when the leukocyte count was 10,400 per cubic millimeter. After forty-eight hours there was a marked increase in both polymorphonuclear leukocytes and lymphocytes.

The reaction of the monocytes is of interest in this case: In three hours and forty minutes after infection, there was a sudden rise in these cells so that they outnumbered the lymphocytes. In twenty-two hours, macrophages containing phagocytosed erythrocytes could be seen in the blood smears. From that time on their presence in the differential count was considerable, and at the seventy-fifth hour following the infection they made up almost one half of the total leukocytic count, outnumbering by far the lymphocytes. The animal died at the end of the fourth day, although before death there occurred a very marked rise in the following white blood cells: granulocytes, lymphocytes and monocytes. The leukopenia, which developed soon after the infection, was not markedly pronounced, being followed by an increased production of granulocytes and by a marked histiomonocytosis. Examination of the bone marrow after death of the animal showed areas of patchy necrosis involving mostly the granulocytic elements. These areas of degeneration were surrounded by masses of proliferated reticular cells obliterat-

2. Lewis, Paul A., and Shope, Richard E.: The Blood in Hog Cholera, J. Exper. Med. **50**:719, 1929.

ing the normal structure of the marrow (fig. 2). The erythropoietic cells and the megacaryocytes were practically unaffected.

Rabbit 170 (fig. 3).—This animal, infected with 300,000,000 organisms, died six days following the infection. A progressive decrease in white blood cells was noticed, beginning a few minutes after infection, reaching its height at four hours and forty minutes. Reduction in granulocytes was, however, most marked at one hour. On the day following the administration of bacteria, there was a marked rise in granulocytes due to bone marrow activity as evidenced by the marked increase in the number of immature polymorphonuclear leukocytes. At fifty-two hours and in five days there were again decreases in granulocytes, but these were transitory. Just before death, a sudden rise in leukocytes, due principally to the rise in granulocytes, occurred. The lymphocytes were ordinarily diminished during most of the course of the disease. Here, too, as in rabbit 169, the monocytic response became important. Thus, beginning at twenty-two hours, there was a noticeable increase in

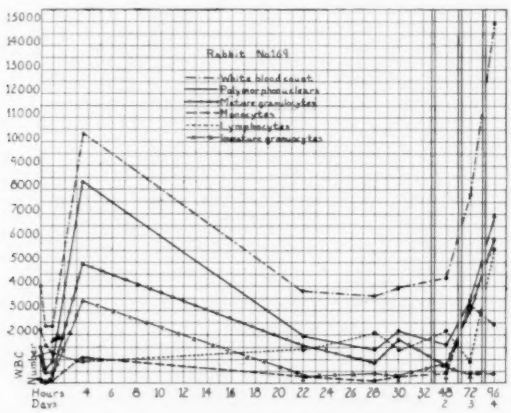

Fig. 1.—Blood of rabbit 169.

these cells, and in the last two days of the rabbit's illness they tended to outnumber the lymphocytes. On the fifth day following infection, they represented exactly one half of the total number of the white blood cells. Phagocytic macrophages appeared in large numbers during this time. The increase in monocytes and the slight lymphocytopenia were reminiscent of the increased ratio of monocytes to lymphocytes, which has been emphasized by Sabin and her associates[3] in infection with tubercle bacilli. Postmortem examination of the bone marrow showed changes not unlike those found in the previous animal.

Rabbit 173 (fig. 4).—This animal, infected with 500,000,000 organisms, died eleven days after the infection. There was a rapid fall in the total number of the white blood cells from the original of about 5,000 to 2,000 per cubic millimeter in two hours and thirty-five minutes. This was due chiefly to a fall in granulocytes, although the lymphocytes also became reduced. At three hours and fifty minutes there was a sudden increase in immature polymorphonuclear leukocytes which

3. Cunningham, R. S.; Sabin, F. R.; Sugiyama, S., and Kindwall, J. A.: The Rôle of the Monocyte in Tuberculosis, Bull. Johns Hopkins Hosp. **37**:231, 1925.

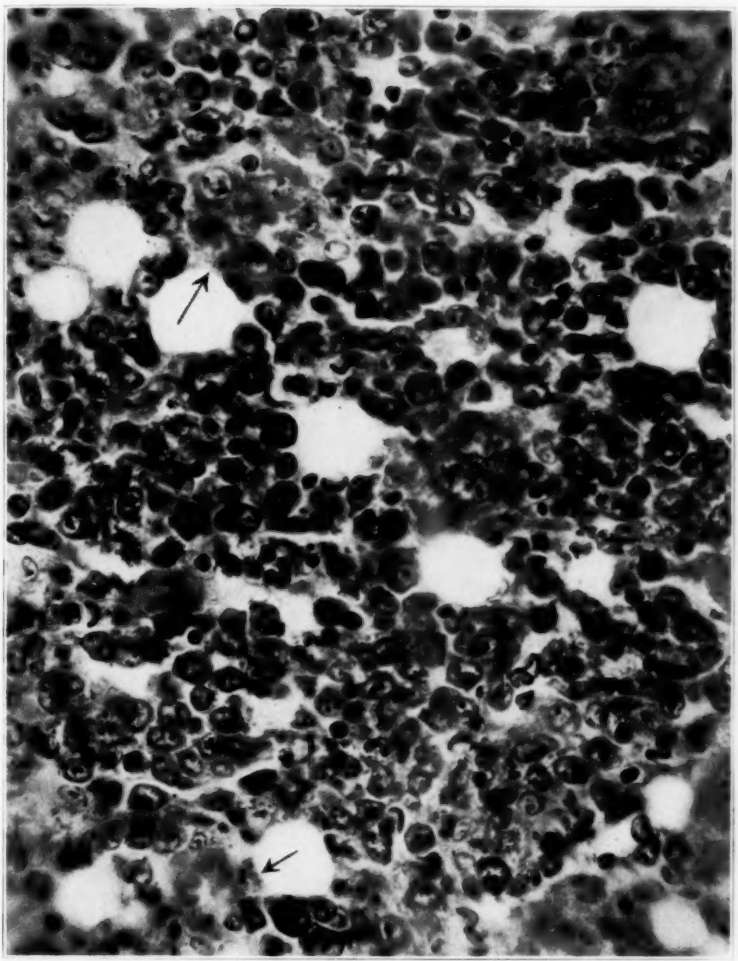

Fig. 2.—Small areas of necrosis of the bone marrow (indicated by arrows) surrounded by proliferated reticular cells. The erythroblastic tissue and the megacaryocytes are not affected. Hematoxylin and eosin; × 600.

reached its peak in five hours and twenty minutes. This resulted in an increase in the total number of the white blood cells. At twenty-four hours, however, there was evidence that the granulocytes were again becoming diminished, and at forty-eight and seventy-two hours, respectively, there was a marked diminution in these cells. At twenty-four hours, monocytes and histiocytes became markedly increased, so

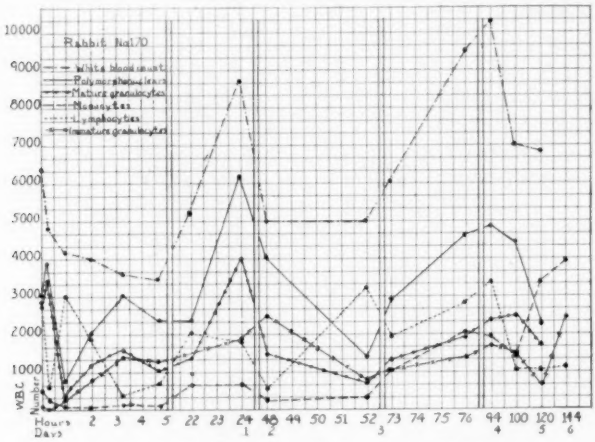

Fig. 3.—Blood picture of rabbit 170.

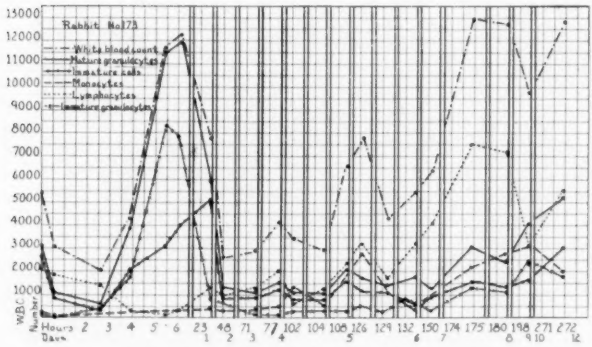

Fig. 4.—Blood picture of rabbit 173.

that at times during this period they outnumbered the lymphocytes. The latter cells, which had been normal or somewhat reduced in number, became definitely increased in number beginning with the fifth day following infection, reaching their peak on the seventh day. On the twelfth day following infection, the animal died, the leukocyte count being fairly high and all the leukocytic elements being well represented. Postmortem examination of the bone marrow showed islands of necrosis of the leukopoietic elements.

Rabbit 181 (fig. 5).—This rabbit was infected with 100,000,000 micro-organisms. It recovered from the infection and was observed over a period of twenty days. One hour after infection there was a marked fall in the white blood cells due mainly to a decrease in the granulocytes, although the lymphocytes also were diminished. This was followed by an outpouring of immature polymorphonuclear leukocytes after two hours, resulting in a marked rise in the white blood cells. Again on the second day, a secondary fall in the white count took place due chiefly to diminution in granulocytes. Monocytes began definitely to increase in about six hours and from that time represented a large factor in the differential count, out-

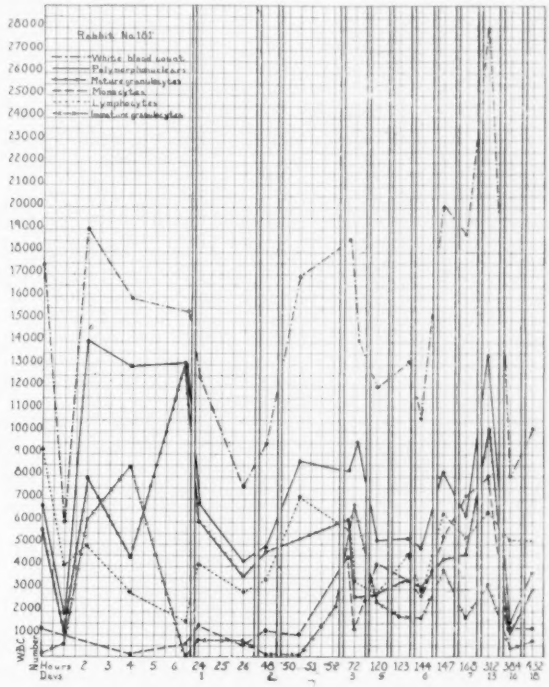

Fig. 5.—Blood picture of rabbit 181.

numbering the lymphocytes at various times from one hundred and twenty hours on. Lymphocytes began to increase consistently after the first monocytic rise, finally reaching a fairly constant level of 5,000 per cubic millimeter. In this animal there was first leukopenia due to granulocytopenia and lymphocytopenia. This was followed by an increase in immature polymorphonuclear leukocytes, resulting in a normal or an increased count of the white blood cells which in turn was followed by an increase in monocytes and finally by an increase in lymphocytes.

Summary.—The animals of this group, given a relatively small dose of bacteria, developed an initial leukopenia and granulocytopenia within three hours. This was soon followed by an outpouring of immature

granulocytes and in about twenty-four hours by a marked increase in monocytes and histiocytes. The latter frequently contained phagocytosed material. Lymphocytosis developed after several days. The animals died usually in from four to eleven days after infection, one animal remaining alive after twenty days. Postmortem examination of the bone marrow showed patchy areas of necrosis with areas of proliferation.

Group II (large doses).—*Rabbit 164* (fig. 6).—This animal was given an injection of 2,000,000,000 bacteria and killed about thirty minutes after the infection. Within a period of twenty minutes, the original count of 4,500 white cells per cubic millimeter dropped to 1,400 per cubic millimeter. A differential count of the white corpuscles revealed that at this time the granulocytes numbered 182 per cubic millimeter, as compared with the original count of 3,150 per cubic millimeter.

Rabbit 165 (fig. 6).—This rabbit was given an injection of 4,000,000,000 microorganisms and killed about twenty minutes later. Counts made before the injection

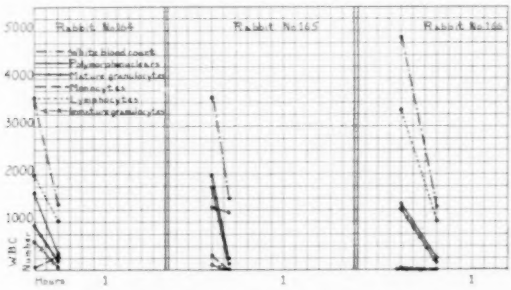

Fig. 6.—Blood picture of rabbits 164, 165 and 166.

showed that the leukocytes numbered 3,600 per cubic millimeter with 1,944 granulocytes. Fifteen minutes following the infection, the white cell count was 1,500 per cubic millimeter with only 285 granulocytes. There was no change in the number of lymphocytes.

Rabbit 166 (fig. 6).—This animal was inoculated with 5,000,000,000 bacteria and killed about forty minutes later. Before injection, the total white cell count of this animal showed 4,800 leukocytes with 1,368 granulocytes per cubic millimeter. Within thirty minutes after injection there occurred a sharp fall of the leukocytes which numbered only 1,300 per cubic millimeter; the granulocytes had almost entirely disappeared from the circulation, and there also was some diminution in the number of the lymphocytes.

Rabbit 174 (fig. 7).—This animal was given an injection of 5,000,000,000 bacteria. The normal count of 8,200 white cells per cubic millimeter dropped to 4,200 within forty-five minutes, and the preinjection count of granulocytes of 3,854 per cubic millimeter fell to 2,500. Seventy minutes after the injection, there was halving of the normal white cell count, and the granulocytes numbered 840 per cubic millimeter. In the following minutes, the leukocytic count was showing

rapid diminution, the total white cell count numbering 1,400 per cubic millimeter with only 415 granulocytes. At this period a few immature granulocytes began to appear in the circulation. There was a very moderate diminution in the number of the lymphocytes.

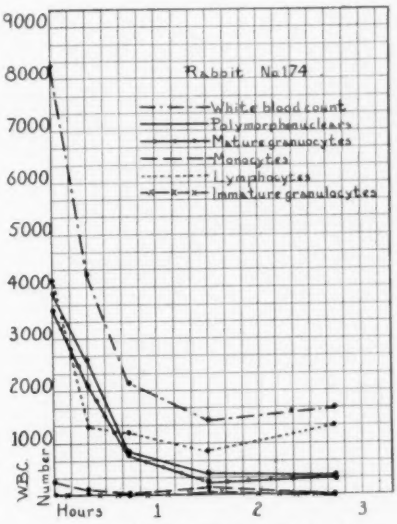

Fig. 7.—Blood picture of rabbit 174.

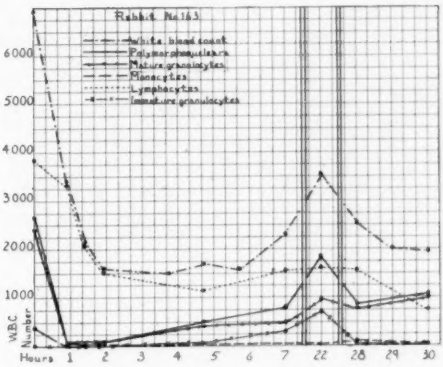

Fig. 8.—Blood picture of rabbit 163.

Rabbit 163 (fig. 8).—This rabbit received an injection of 4,000,000,000 bacteria. The normal white cell count was 7,000 per cubic millimeter, the differential count of these showing 40 per cent granulocytes, 55.6 per cent lymphocytes and 4.4 per cent monocytes. Thirty minutes after infection the leukocytic count fell to 3,400 per cubic millimeter, this drop resulting from almost complete disappearance (to 1.5

per cent) of the granulocytes from the circulation. Lymphocytes were present in normal absolute numbers, their relative value being of course high (98 per cent). Eighty minutes after injection the leukopenia was still greater, the white blood cells numbering only 2,200 per cubic millimeter. Only an occasional degenerated granulocyte could be found in the blood smears at this stage. Lymphocytes, too, were beginning to be diminished in number, whereas the monocytes were altogether absent in the circulation.

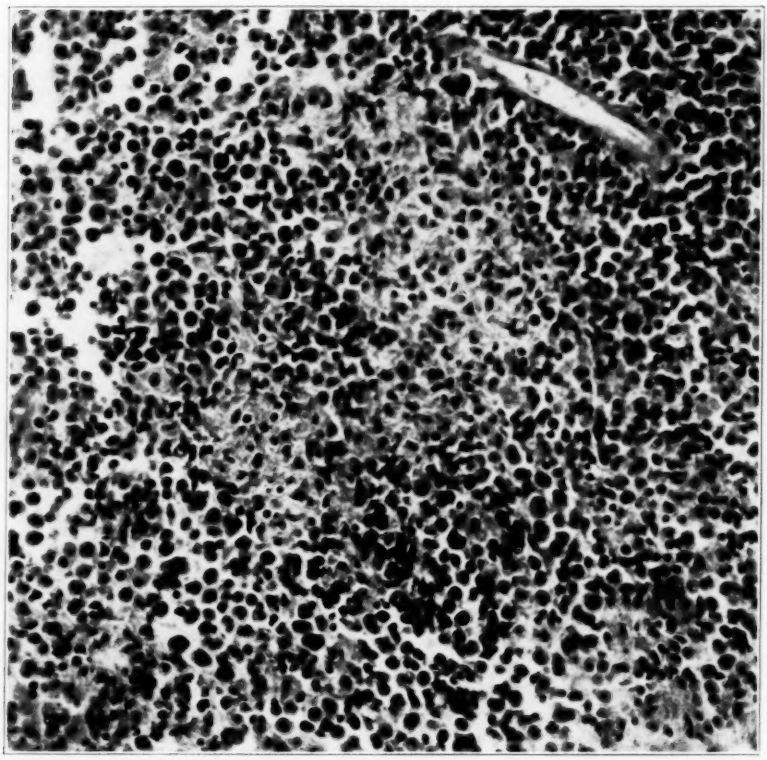

Fig. 9.—Patchy necrosis of the bone marrow with no pathologic changes in the erythroblastic tissue. Hematoxylin and eosin; × 300.

Two hours after the infection, the leukocyte count was very low, 1,025 per cubic millimeter, the relative proportion between the white cells being practically unchanged. The lowest leukocytic count (1,500 per cubic millimeter) was noticed three hours and forty-five minutes after the infection. The first rise in white cells took place about five hours after the infection, at which time the white blood cell count equaled 1,725 per cubic millimeter with a granulocytic count of 30 per cent. There was no appreciable change within the next hours. At twenty-two hours the number of the white cells had risen to 3,600 per cubic millimeter, and there was

evidence of a beginning regenerative attempt on the part of the bone marrow; thus of 52.5 per cent of granulocytes, 24.5 represented immature forms. Of the leukocytes seen at this period in the circulation, about 8 per cent had the appearance of plasma cells. It is interesting that at this time the granulocytes began to show marked "toxic" changes, as evidenced by vacuolization of the cytoplasm and complete disintegration of the cell body. The foregoing rise in leukocytes was, however, transient. At twenty-eight hours, the leukocytic count revealed only 2,600 per cubic

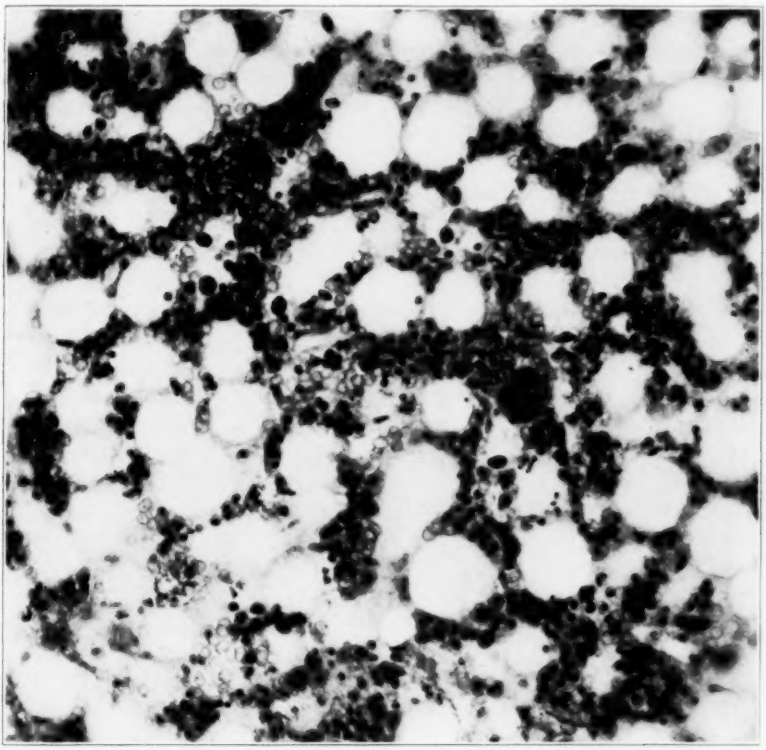

Fig. 10.—Normal islands of erythroblastic tissue with almost complete disappearance of granulocytic cells in an animal infected with a large dose of bacteria. Hematoxylin and eosin; × 300.

millimeter with very low counts of the granulocytes. Monocytes, which had not been seen in the circulation during the entire period of infection, began at this time to appear in the blood, numbering about 5 per cent of the total white cell count. Most of them were of the histiocytic variety. Postmortem examination of the bone marrow revealed a marked necrosis confined to the leukopoietic system. However, even in the presence of this marked infection, areas of normal and regenerating marrow could be seen in places (figs. 9 and 10).

Summary.—These animals, infected with an overwhelming dose of bacteria, rapidly developed severe leukopenia and almost complete agranulocytosis. There was but little tendency toward regenerative activity on the part of the granulocytes, and monocytosis did not develop to any appreciable extent. The animals died usually within forty-eight hours after infection. The bone marrow, post mortem, usually showed but little evidence of regenerative activity.

COMMENT

It may be seen that under the conditions of our experiments the reaction of the granulocytic (myelopoietic) apparatus was closely related to the gravity of the infection. Thus animals infected with small doses of micro-organisms (figs. 1, 2, 3, 4 and 5) in which the infection was left to run its natural course lived for four, six, eleven and twenty days, respectively, following inoculation, animal 181 surviving the experiment, being well after twenty days. Although the time of appearance of the lowest leukocytic and granulocytic counts varied among individual animals from fifteen minutes to four hours and forty minutes, the average time was two hours. However, definite evidence of a marked increase in the production of granulocytes was seen in from three to four hours. It may be said that the time for the appearance of large numbers of immature polymorphonuclear leukocytes, which at times reached the figure of from 8,000 to 9,000 per cubic millimeter, was surprisingly constant, varying within the narrow limits of three and four hours. This increase in immature granulocytes resulted, of course, in a marked increase in the total number of the white blood cells. This group of animals was distinguished from the others, not only by the longer course of the disease, but chiefly by the marked monocytosis they developed. An increase in monocytes, which was generally associated with the presence of histiocytes as well, took place in from four to twenty-four hours following the inoculation, reaching its peak in from fifty-two to one hundred and forty-four hours after infection. At this period monocytes sometimes outnumbered lymphocytes, being present in numbers varying from 3,000 to 8,000 per cubic millimeter. It is interesting that once the monocytic rise was at its peak, this tended to be maintained, so that the monocyte-lymphocyte ratio was often 1 or even over 1. At this period of the infection, intensely phagocytic macrophages could often be seen. At the height of the monocytic rise there also occurred a sharp increase in lymphocytes, this taking place in from fifty to one hundred and seventy-four hours following the infection. However, this lymphocytosis had a tendency to diminish during the course of the disease.

The blood picture in this group of animals can therefore be divided arbitrarily into five stages: (1) stage of leukopenia, due to diminution in granulocytes and lymphocytes; (2) stage of marked increase in immature granulocytes; (3) stage of monocytosis and histiocytosis; (4) stage of lymphocytosis, and (5) stage of normality.

The duration of the disease in rabbits that received large doses of bacteria was about forty-eight hours. In these animals leukopenia resulting from a granulocytopenia developed within a short period, varying from fifteen to thirty minutes following the infection. The total white blood cell count after infection never rose above 3,600 per cubic millimeter, being generally below that figure. In an occasional instance granulocytes were reduced to the low figure of 22 per cubic millimeter. Immature forms of granulocytes never rose above 800 per cubic millimeter. There was at no time a rise in either monocytes or lymphocytes. Likewise no changes were observed in the red cells. In brief, only the first of the stages, that is, of leukopenia (neutropenia) without any signs of regeneration, was present in the blood of this group of animals during the entire course of their illness. The pathologic changes found in the visceral organs resembled those seen in sepsis in general. In the bone marrow, changes were obvious even in those animals that were killed thirty minutes after the infection took place. As in persons with agranulocytosis, the disease was apparently confined to the myelopoietic system only, as evidenced by various degrees of degeneration and wide areas of necrosis. Islands of normally appearing normoblasts could often be seen surrounded by necrotic leukopoietic tissue. The megacaryocytes, too, showed no appreciable changes.

In summary, a relatively small dose of bacteria produced a slight leukopenia followed by an intense regeneration in the bone marrow (fig. 11), this being followed by a histiomonocytosis in the circulating blood and later by a lymphocytosis. With a moderately large dose of bacteria, leukopenia, which was not very intense, developed more quickly. Usually a slight regenerative activity was seen and at times a slight monocytosis. An overwhelming dose of micro-organisms brought about a very rapid leukopenia resulting from an agranulocytosis with areas of necrosis of the bone marrow. No regeneration of bone marrow (fig. 12) occurred up to the death of the animal.

Are there any similarities between agranulocytosis as seen in man and that resulting from sepsis in the rabbit?

In human beings, as noted in the introductory paragraphs, the disease agranulocytosis (Schultz) is characterized by: (1) clinical signs, such as necrosis of the gums, tonsils, buccal and gastric mucosae, vulva and vagina; (2) a hematologic picture evidenced by the diminution or almost

complete disappearance of granulocytes from the circulation, and (3) the absence of a hemorrhagic diathesis. It is characteristic in that the etiology of this disease is unknown, it being thus included in the category of "essential" malignant neutropenia. Schultz was of the opinion that agranulocytosis is a disease per se, being caused by a specific agent that has a particular affinity for the myeloid system. Other

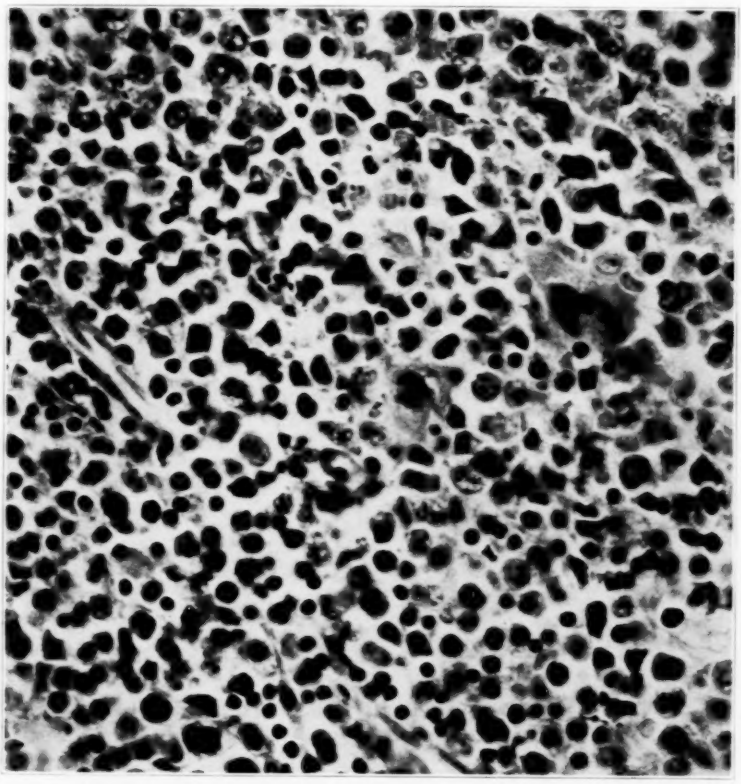

Fig. 11.—Intense regeneration of bone marrow at the border of a necrotic area. There are no pathologic changes in the erythrocytic elements. Hematoxylin and eosin; × 600.

workers regard the malady as a symptom complex of which sepsis is the underlying condition. The hypothesis also has been expressed that it is a form of hyperergic inflammation (allergy) in which the bone marrow is the locus minoris resistentiae. This hypothesis will not be considered here since the study was performed on normergic (anergic) animals.

Remaining to be discussed are: (1) the necrotic changes of the mucous membranes and (2) the hematologic picture.

As referred to, the condition was isolated as an "entity" because in addition to the agranulocytosis there occurred a necrotizing process in the mucous membranes which was said never to happen in other diseases. However, as has also been noted by other writers,[4] necrosis

Fig. 12.—Focal necrosis of bone marrow. Hematoxylin and eosin; × 300.

of the buccopharyngeal mucosae occurs in other maladies such as acute leukemia, hemorrhagic diathesis and also sepsis.

Moreover, it has been observed that the occurrence of typical cases of agranulocytosis is not necessarily accompanied by necrotizing

4. Barta, T., and Erös, G.: Sepsis und Blutbildung, Virchows Arch. f. path. Anat. **272**:313, 1930. Blumer, G.: Agranulocytic Blood Picture in Conditions Other Than Angina, Am. J. M. Sc. **179**:11 (July) 1930.

lesions of the mucous membranes. Thus, in a series of patients studied at the Beth Israel Hospital,[5] two patients with a characteristic clinical course and a hematologic picture of this disease failed to show necrosis of mucous membranes. One patient developed necrosis of the cutaneous tissues in the antecubital fossae of the arms following a needle puncture. The history of this patient is as follows:

A woman, aged 41, who had been an invalid for several years, developed an acute infection of the upper respiratory tract eight months prior to admission to the hospital. Since that time she had had constant headaches. Two months before entry she had swelling and stiffness of the knees. One week before admission she became very ill with high fever and slight delirium.

At the hospital she showed a lateral nystagmus of both eyes. The lymphopoietic and erythropoietic systems showed no changes. Her leukocytic count varied between 600 and 1,900 per cubic millimeter. The granulocytes gradually disappeared. A specimen of bone marrow taken for biopsy showed almost complete absence of granulocytes, only a few myeloblasts being found. Following a blood transfusion, the incision wound did not heal, becoming thick and edematous. A white sloughing area of necrosis finally developed. Aerobic blood cultures were sterile, but anaerobic cultures showed a streptococcus. The patient died seventeen days after the onset of the illness.

Here, then, is a case of agranulocytosis with characteristic changes in the circulating blood as well as in the bone marrow in which gangrenous lesions of the mucous membranes were absent. In fact, since the original description of this disease, the experience of most writers is that this condition is not as "rigid" as described in the early reports and that this clinical entity abounds in multiple clinical and hematologic nuances.

From a study of the literature and from the cases observed at the Beth Israel Hospital,[5] we gained the impression that from a clinical standpoint, the disease agranulocytosis can be arbitrarily divided into three main groups: 1. Severe cases like those originally reported by Schultz. In these instances the onset of the malady is sudden, with a chill, high fever and a necrotizing angina, jaundice and albuminuria being frequently present. The blood in these patients shows an extreme degree of agranulocytosis, and the bone marrow reveals widespread necrosis of the leukopoietic system. The disease is rapidly fatal. 2. Moderately severe cases in which the disease is more protracted, occasionally ending in recovery. The blood of these patients shows a very marked leukopenia, with a few granulocytes, and very numerous monocytes and histiocytes. In the bone marrow one finds areas of necrosis and signs of active regeneration of the myelopoietic system. The patients may or may not have necrotizing lesions of their

5. Dameshek, W., and Ingall, M.: Agranulocytosis (Malignant Neutropenia), Am. J. M. Sc. **181**:502, 1931.

mucosae. 3. Mild cases of agranulocytosis in which the disease begins insidiously, having a much longer course. In these patients the leukopenia is less intense and the number of granulocytes is higher than in the moderately severe cases. Again the blood smears show that a high percentage of monocytes and macrophages is present in the circulation, which may be regarded as a "recovery stage" (Schilling [6]). The bone marrow, in addition to necrosis, shows a very active power of regeneration of the leukopoietic system. In these cases, too, necrotic lesions of the mucosae may be absent, and the disease ends in most instances in recovery.

It may be seen then that necrotizing lesions of the mucosae may be present in diseases other than agranulocytosis, and that in this disease the lesions of the mucosae are not a conditio sine qua non.

In analyzing the hematologic findings in the animals in our experiments, we find striking similarities between the agranulocytosis resulting from the infection of rabbits with Salmonella suipestifer and that seen in the clinic. Thus the severe cases of the disease in man correspond closely to the reaction seen in the animals of group II which received overwhelming doses of bacteria, causing intense necrosis of the bone marrow without signs of regeneration. There is also a close similarity existing between the "recovery phases" seen in the circulating blood in clinical agranulocytosis and that disclosed in the circulation of rabbits that were infected with relatively small doses of bacteria. Two aspects of these phases of particular interest will be discussed briefly: (1) the occurrence of immature granulocytes in the blood, and (2) the presence of histiocytes (macrophages) in the circulation.

The differential count of the white cells in the present experiments was made according to the method of Arneth as modified by Schilling. In the modification of the latter, four groups of polymorphonuclear leukocytes are discriminated, namely, mature or segmented forms, band or "stabkernig" forms, young forms and myelocytes. In our study the last three varieties of cells were grouped together as immature polymorphonuclear leukocytes (immature cells in the charts).

The significance of an increase in these cells in the circulating blood following an initial granulocytopenia obviously represents a rapid regenerative activity on the part of the white cells of the bone marrow in response to injury. In the experiments herein presented, this was brought out in the animals that were killed at the height of increase in the number of the immature granulocytes (rabbits 175, 176, 177, 178 and 179). The bone marrow in these rabbits showed, in addition to necrosis, areas of intense regeneration.

6. Schilling, V.: The Blood Picture, St. Louis, C. V. Mosby Company, 1929.

Paradoxical as it may appear, immature polymorphonuclear leukocytes may be present in increased numbers in the circulation in instances of almost complete necrosis of the bone marrow. It was thought by Schilling that this resulted from the gradual discharge of the remnants of the immature leukocytes of the bone marrow. In these instances a steady decrease in the total number of the polymorphonuclear leukocytes is observed instead of the customary rise in these cells which is found in conditions with regenerative activity of the marrow. These two types of reactions were designated by Schilling [6] as the "regenerative" and "degenerative" shifts of the white blood cells. It is interesting then that what was advanced by Schilling as a mere hypothesis has been borne out in our experiments (rabbits 163, 171, 167 and 180).

In regard to the histiomonocytosis observed in the blood stream, it is interesting that whereas the monocyte is seen normally in the circulating blood, the histiocyte (macrophage) is present in the circulation only in pathologic conditions.[7] Simpson [8] and others were able to induce experimentally an invasion of the blood by these large phagocytic cells, and one of us [9] (Dr. Dameshek) observed a marked increase in macrophages in the blood in the convalescent stage of various infectious diseases, in certain chronic infections such as syphilis, and also in the leukemias.

In the present investigation a marked increase in the monocytes and histiocytes (fig. 13) occurred regularly in the blood of those rabbits that received relatively small doses of bacteria (group I). A subsequent study of the tissues of these animals revealed a marked proliferation of the cells of the reticulo-endothelial (macrophage) system. It is thus apparent that the blood was flooded with these cells as a result of the overactivity of the mesenchymal defensive apparatus. The "stage of defense" may, however, be only ephemeral, as can be seen in many of our experiments in which the animals subsequently died. In two patients with agranulocytosis recently observed in the clinics of this hospital, the stage of recovery was likewise accompanied by an invasion of the blood with macrophages which numbered about 60 per cent of the total count of the white blood cells. In these cases the "defense" was apparently efficient since the patients recovered from their illness. This may possibly be due to the fact that: (1) experimental infection is as a rule more severe than that observed clinically in man, and (2) in experimental inoculation which is performed in

7. Maximow, Alexander A.: The Macrophages or Histiocytes, in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, Inc., 1928, vol. 1, p. 427.

8. Simpson, M. H.: The Experimental Production of Macrophages in the Circulating Blood, J. M. Research **53**:77, 1922.

9. Dameshek, W.: The Appearance of Histiocytes in the Peripheral Blood, Arch. Int. Med. **47**:968 (June) 1931.

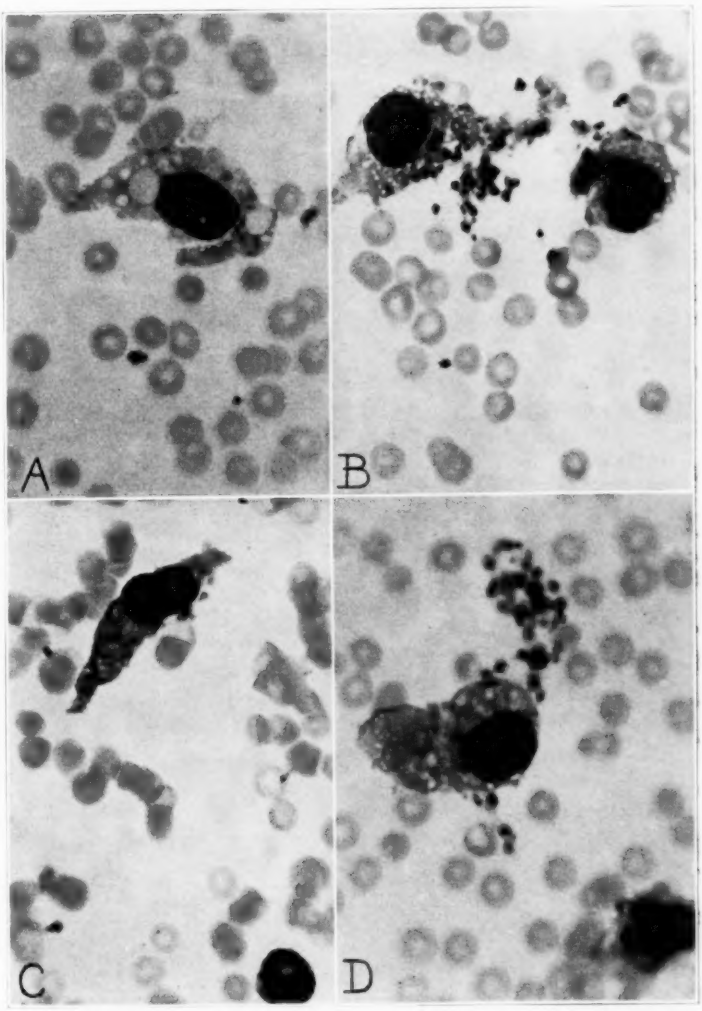

Fig. 13.—Macrophages (histiocytes) in the circulating blood. *A*, phagocytosis of red cells by a macrophage. Hematoxylin and eosin; × 850.

normergic (anergic) animals the "dose" of the infectious material (other conditions being equal) plays apparently the dominating rôle, whereas in man "constitutional" factors as well enter into play.

CONCLUSIONS

The purpose of the present study has been to determine the possible similarity between the blood picture as seen in agranulocytosis in man and that found in a form of experimental sepsis in rabbits.

The results have shown that there are close similarities between the agranulocytosis resulting from the hematogenous infection of rabbits with Salmonella suipestifer and that observed in cases of agranulocytic angina in man. Thus, the reaction in severe cases of human agranulocytosis corresponds to that of the animals that received overwhelming doses of bacteria, i. e., a persistent neutropenia and an intense necrosis of the bone marrow without signs of regeneration. A close similarity likewise exists between the "recovery phase" seen in the circulating blood in clinical agranulocytosis and that disclosed in the circulation of rabbits that were infected with relatively small doses of bacteria, i. e., a marked histiomonocytosis.

Incidentally, Schilling's clinical concept of "regenerative" and "degenerative" types of polymorphonuclear "shift" was confirmed by these experiments.

SOME IMMUNOLOGIC ASPECTS OF LEUKEMIA*

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Investigations of Carrel and Ebeling [1] into the proliferative activity of blood leukocytes in tissue cultures have shown that homologous serum possesses a marked antiproliferative effect on polymorphonuclear leukocytes and lymphocytes. The first mentioned type of white blood cell disappears entirely from the culture in a few days, and the latter type in from one to two weeks. By a process of physiologic selectivity a pure culture of mononuclear cells remains, which can be kept alive for several months. Carrel and Ebeling concluded from this observation that homologous serum may possess a similar action in vivo, because the number of leukocytes would increase indefinitely if serum were not endowed with this property. They added that probably the increase of the growth-inhibiting action of serum in the course of life determines a decrease in the activity of the white cells and modifies their secretions. They suggested, moreover, that possibly this is one of the mechanisms by which the profound changes are brought about by age in blood serum, and that it may be related to certain diseases in this period, such as cancer.

It occurred to us that perhaps a disturbance in the antiproliferative activity of the blood serum might be the cause of the enormous increase of leukocytes in leukemias. The correctness of this supposition required that leukemias should be characterized by a decrease or a lack of the antiproliferative qualities of the leukemic serum, allowing an excessive and unimpeded proliferation of leukocytes. To put this theory to a test, the effects of normal and of leukemic plasma on the proliferative activity of normal and leukemic leukocytes were studied with the tissue culture method.

EXPERIMENTS

The donor of the leukemic blood was a woman, 53 years of age, who had been under clinical observation by Dr. E. Ellice McDonald for more than a year preceding our experiments. Her spleen was considerably enlarged, extending laterally somewhat beyond the umbilicus and reaching well into the pelvis. Her blood picture at the beginning of our

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* From the Cancer Research Laboratories, University of Pennsylvania, Graduate School of Medicine.

1. Carrel and Ebeling: J. Exper. Med. **36**:365, 1922.

experiments was as follows: hemoglobin, 50 per cent (Sahli); erythrocytes, 3,500,000; leukocytes, 750,000; neutrophilic leukocytes, 50 per cent; myelocytes, 23 per cent; myeloblasts, 6 per cent; lymphocytes, 8 per cent; monocytes, 2 per cent; eosinophils, 7 per cent, and basophils, 4 per cent. The normal blood was taken from a healthy man, 36 years old, with normal observations on the blood. The blood needed for the experiments was obtained by venous puncture, heparin being used as an anticoagulant. The method of Carrel and Ebeling [1] was employed to secure the plasma and the buffy, leukocytic coat. The latter was cut into small pieces, which were explanted into a drop of plasma, the slide method of tissue culture being used. There were four sets of *tissue cultures*, each containing twelve slides:

1. Normal leukocytes in normal plasma.
2. Normal leukocytes in leukemic plasma.
3. Leukemic leukocytes in normal plasma.
4. Leukemic leukocytes in leukemic plasma.

The experiments were repeated three times to check the results obtained. These were as follows: It was uniformly observed that the growth and emigration of the normal leukocytes in the leukemic plasma lagged behind those seen in normal plasma cultures during the first twenty-four hours of incubation. This difference became less distinct after forty-eight hours and was negligible thereafter. If leukemic leukocytes were used in this combination, the retarding effect was less marked than in the previous experiment during the first twenty-four hours and was only relatively slight in the forty-eight hour cultures. In the latter observation our results are in agreement with and substantiate those obtained by Hirschfeld.[2]

As the leukemic patient was 53 years of age and the donor of the normal blood only 36 years old, it appeared possible that the difference in growth activity observed might have been caused by the difference in age, resulting in a higher antiproliferative quality of the plasma from the leukemic patient. To exclude this possibility, an identical set of experiments was run, a man 56 years of age being used as the source of normal plasma and leukocytes. The results were the same, but differed somewhat in degree, as the proliferative activity of the normal leukocytes in the normal plasma was not quite so good in this experiment as in the previous experiments in which the blood came from a younger person. The differences in the growth activity of the normal leukocytes in normal plasma compared with that in leukemic plasma were therefore less marked, but still definitely evident.

2. Hirschfeld: Folia haemat. **34**:39, 1927.

The conclusions drawn from these investigations are as follows: Leukemic blood does not contain a decreased amount of antiproliferative substance, but has rather a slightly increased amount, if proper consideration is given to the age factor. The enormous increase of leukocytes in leukemias can therefore not be attributed to a disturbance in the physiologic antileukocytic quality of blood serum.

The subsequent qualitative study of the cultures in regard to the type of leukocytes that emigrated from the normal and the leukemic plasma cultures respectively supplied the evidence by which the quantitative differences demonstrated could be properly explained. It was found that the emigration of polymorphonuclear leukocytes was more or less markedly suppressed in the leukemic plasma cultures after twenty-four hours of incubation. As this type of cell disappears normally from the cultures in the course of seventy-two hours, one had to expect that the quantitative differences would decrease after prolonged incubation. This expectation was substantiated by our observations already mentioned. A decreased emigration of polymorphonuclear leukocytes in cultures of normal human leukocytes in leukemic plasma must be regarded as the cause of the quantitative differences observed between the growth activities of these cells in normal and leukemic plasma respectively. The emigration and growth of lymphocytes and monocytes were apparently not interfered with by the plasma of a myeloid leukemia.

Leukemic leukocytes had been cultured previously in normal and leukemic plasma by Awrorow and Timofejewskij,[3] Hirschfeld [2] and Parker and Rhoads.[4] We can substantiate their results and have nothing to add to them. The transformation of mononuclear leukocytes into fibroblast-like cells was especially evident and frequent in cultures of leukemic leukocytes in normal plasma after seventy-two hours of growth.

If we attempt to interpret our observations and try to explain them by processes in the organism which might be responsible for the phenomena observed in the tissue cultures, we are inclined to believe that the massive destruction of myeloid cells in the system of the leukemic patient results in an increase of the antiproliferative qualities of the serum directed against the myeloid cells.

Applying this conception, we attempted to produce an antileukocytic serum by repeated injections of leukemic leukocytes into the veins of the ears of rabbits. The following technic was employed: The buffy coat of 10 cc. of venous blood from the leukemic patient was washed in sterile Ringer's solution, to remove the adherent erythrocytes as much as possible, and then ground in a mortar with a small amount of Ringer's solution. When a more or less homogeneous mass was obtained, from 15 to 20 cc. of Ringer's solution was added. The suspension had a slightly

3. Awrorow and Timofejewskij: Virchows Arch. f. path. Anat. **216**:184, 1914.
4. Parker and Rhoads: Am. J. Path. **4**:167, 1928.

pink color. Immediately after preparation one half of it was injected into the veins of the ears of the rabbit, while the remaining portion was kept in the icebox and was injected three days afterward. The first rabbit used received eight injections.

Fig. 1.—Leukemic leukocytes in the plasma of a rabbit given eight injections; twenty-four hour culture; no emigration.

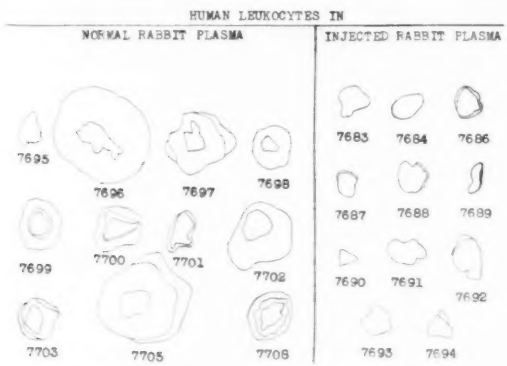

Fig. 2.—Twenty-four hour cultures traced with the Edinger projectograph.

When normal and leukemic leukocytes were cultured in the plasma of the rabbit thus treated, it was seen that the plasma had a marked inhibiting effect on the emigration of leukocytes. There was either no emigration or only a very slight one. After three days of incubation a

lysis of the plasma around the explant was observed, which was apparently due to the action of proteolytic enzymes freed from the dying leukocytes. There was no evidence of leukocytolysis. Control experiments with leukocytes in normal rabbit plasma showed an abundant leukocytic emigration (fig. 2).

The plasma of the rabbit given injections of leukemic leukocytes had, according to these observations, acquired an antileukocytic quality which proved, however, to be of a nonspecific nature, as not only the emigration of leukemic leukocytes, but also that of normal human leukocytes was impaired.

In a second series of experiments we attempted to determine the antileukocytic titer of the plasma by the tissue culture method, using a rabbit which had received five injections of leukemic leukocytes. The following sets of cultures, each consisting of six slides, were made:

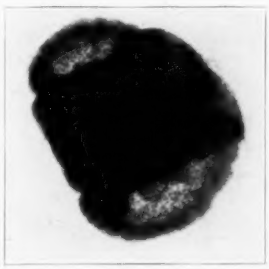

Fig. 3.—Normal leukocytes in the plasma of a rabbit given five injections; twenty-four hour culture; scanty emigration, with a peripheral ring of agglutinated leukocytes.

1. (*a*) Normal leukocytes in the plasma of the rabbit given the injections.
   (*b*) Normal leukocytes in normal rabbit plasma.
2. (*a*) Normal leukocytes in three parts of plasma from the rabbit given the injections plus one part of Tyrode's solution.
   (*b*) Normal leukocytes in three parts of normal rabbit plasma plus one part of Tyrode's solution.
3. (*a*) Normal leukocytes in two parts of plasma from the rabbit given the injections plus two parts of Tyrode's solution.
   (*b*) Normal leukocytes in two parts of normal rabbit plasma plus two parts of Tyrode's solution.
4. (*a*) Normal leukocytes in one part of plasma from the rabbit given the injections plus three parts of Tyrode's solution.
   (*b*) Normal leukocytes in one part of normal rabbit plasma plus three parts of Tyrode's solution.

The results obtained are as follows: There was a definite impairment in the emigration of leukocytes from the explants in the leukemic

Fig. 4.—Normal leukocytes in the plasma of a rabbit given five injections; twenty-four hour culture; moderate emigration, with a peripheral ring of agglutinated leukocytes; plasma diluted 50:50 with Tyrode's solution.

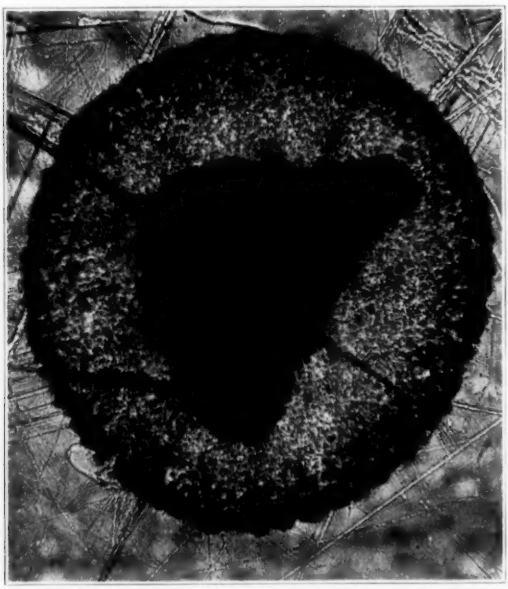

Fig. 5.—Normal leukocytes in the plasma of a rabbit given five injections; twenty-four hour culture; good emigration, with a peripheral ring of agglutinated leukocytes; plasma diluted 25:75 with Tyrode's solution.

rabbit plasma, as compared with the emigration present in the corresponding leukocytic cultures in normal rabbit plasma. This effect decreased in direct proportion to the concentration of the leukemic rabbit plasma present in the medium. There was no emigration evident in 50 per cent of the cultures in concentrated leukemic rabbit plasma. It was, moreover, noticed that the emigration zone was much denser in the cultures with leukemic rabbit plasma than in those with the normal rabbit plasma, becoming, however, less dense with the decreasing concentration of the leukemic rabbit plasma in the culture medium. We observed, furthermore, that the cultures of the leukemic rabbit plasma showed a sharply defined white ring at the periphery of the emigration zone. The ring consisted of agglutinated leukocytes. The emigration of leukocytes stopped, in general, rather abruptly at this ring. Leukocytes were only

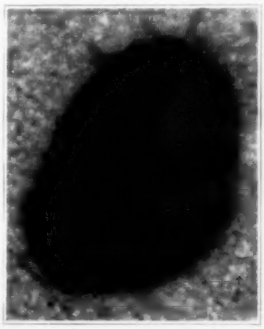

Fig. 6.—Normal rabbit plasma with normal leukocytes; no agglutination; very good emigration.

occasionally found peripherally from this ring in the plasma. The emigration of the leukocytes in normal rabbit plasma was, in strong contrast to the observation just described, abundant and indistinctly defined, and seemed to fade away into the plasma.

It is evident from these observations that a rather accurate titration of the antileukocytic qualities of a serum can be obtained by the tissue culture method. Strongly antileukocytic serums will inhibit the emigration of leukocytes completely, while weakly antileukocytic serums will allow only a restricted emigration. The toxic effect of the antileukocytic plasma on the cells is evident from the positive nuclear stain, especially in the agglutinated leukocytes, after the addition of neutral red, showing that the majority of the cells in the emigration zone are dead. The great significance of these observations may be demonstrated

by the fact that previous investigators have unsuccessfully attempted to determine the antileukocytic titer of such serums by agglutination, precipitation or complement-fixation reactions, while the existence of a leukocytolysis has never been shown beyond any doubt. These authors used bone marrow (Besredka,[5] Flexner,[6] Bunting[6]), spleen or lymph node (Metschnikoff,[7] Funk,[6] Flexner[6]), aseptic purulent exudates (after intraperitoneal injections of aleuronat [Gladin[8]] or injections of staphylococcus vaccine [Bierry[9]]) as the source of leukocytic supply. The tissue culture method must be regarded at the present time as the best procedure for the demonstration and titration of cytotoxins. It has been successfully used for this purpose by Lambert,[10] Foot,[11] Lumsden[12] and Kimura.[13]

Encouraged by the good results obtained with the antileukocytic plasma in tissue cultures, we gave our patient who had supplied us with leukemic leukocytes several subcutaneous injections of antileukocytic plasma and serum, respectively, in the hope that her blood condition might be benefited thereby. She received in the course of six weeks a total of 30 cc. of antileukocytic plasma and serum. The injections caused general discomfort and pain in the region of injection. The spleen decreased somewhat in size, while the leukocytes increased after the first two injections from 470,000 to 700,000 and the erythrocytes from 2,900,000 to 4,000,000. The differential count remained essentially the same. After the second injection the number of leukocytes started to drop and continued to do so until there were (ten days after the last injection) 512,000. The differential count showed at that time a rather moderate shift to the right with an increase of the mature neutrophilic leukocytes and monocytes. The treatment had to be discontinued after the eighth injection on account of the death of the rabbit which had been the source of the antileukocytic serum.

The results so far obtained with the antileukocytic serum in this case allow the following conclusions: The initial rise of the number

5. Besredka: Die lokale Immunisierung, Leipzig, 1926.

6. Quoted by Fischer: Gewebszuechtung, ed. 3, Munich, Mueller & Steinicke, 1930.

7. Metschnikoff: Ann. Inst. Pasteur **14**:369, 1900.

8. Gladin, quoted by Lindstroem: Acta med. Scandinav. (supp. no. 22), 1927, p. 1.

9. Bierry: Compt. rend. Soc. de biol. **54**:1003, 1902; quoted by Lindstroem (footnote 8).

10. Lambert: J. Exper. Med. **14**:453, 1911.

11. Foot: Centralbl. f. allg. Path. u. path. Anat. **23**:578, 1912.

12. Lumsden: Lancet **1**:383, 1924; **1**:112, 1926; Arch. f. exper. Zellforsch. **6**:206, 1928.

13. Kimura: Arch. f. exper. Zellforsch. **6**:185, 1927; Ztschr. f. Immunitätsforsch. u. exper. Therap. **55**:501, 1928.

of leukocytes was caused partly by the expulsion of leukocytes from the contracting spleen and partly by a stimulating effect of the serum injections on the myeloid tissue. The conclusion as to the latter effect was also confirmed by the appearance of numerous normoblasts in the blood. We had apparently not succeeded in building up a serum in which the antileukocytic qualities outbalanced the normal stimulating action of the serum.

While our results in regard to the therapeutic application of antileukocytic serums were rather discouraging, Lindstroem,[14] whose recent work on this subject came to our attention after our experiments had already reached their final stage, reported such remarkable therapeutic effects of antileukocytic serums in leukemias that the use of properly prepared and titrated antileukocytic serums seems to offer good prospects in the future therapeutic management of these disease conditions. Using a technic similar to ours in the preparation of antileukocytic serums, this investigator succeeded in experiments with various animals (rabbits, cats, monkeys, sheep) in lowering the number of leukocytes in some instances from 8,100 to 50 by the injection of antileukocytic serum. The subsequent examination of the animals dying from the effects of the treatment showed a deficiency of granulocytes in the bone marrow similar to that found in cases of agranulocytosis (Hueper[15]). He did not notice the development of a tolerance for, or the production of, antibodies against the antileukocytic serum. Similar observations on the effect of antileukocytic serums on the number of leukocytes in the blood and bone marrow were made by Yamamoto.[16]

In a series of eleven patients with myeloid leukemia treated with rather large doses (from 10 to 20 cc.) of antileukocytic serum, Lindstroem was able to produce four complete remissions characterized by a return of the number of the leukocytes to a normal level and great general improvement in the condition of these patients. These remissions lasted for several months. He pointed out that remissions of this character do not occur spontaneously in leukemias nor can they be produced by irradiation or by arsenical medication. An aggravation of the condition following the treatment was observed by him in four cases of advanced myeloid leukemia. He stated that the success of the treatment depends on the therapeutic strength of the serum, which he was unable to determine with the methods available to him. He noted, furthermore, that patients in the early stage of the disease are best suited for the serum treatment.

14. Lindstroem: Acta med. Scandinav. (supp. no. 22), 1927, p. 1.

15. Hueper: S. Clin. North America **10**:407, 1930.

16. Yamamoto: Tohoku J. Exper. Med. **15**:324, 1930.

CONCLUSIONS

1. The antiproliferative quality of leukemic plasma is not decreased, but slightly increased, as compared with that of normal human plasma. Myeloid leukemia is therefore not the result of the decrease or absence of this quality of the serum.

2. Myeloid leukemic plasma inhibits the emigration of the granulocytic elements from the explanted clot of blood leukocytes.

3. Antileukocytic serum produced by the intravenous injection of leukemic leukocytes into rabbits impairs the emigrative and proliferative qualities of normal and leukemic leukocytes in tissue cultures.

4. Tissue cultures of leukocytes allow a rather accurate titration of the cytotoxic qualities of an antileukocytic plasma.

5. Isolated favorable therapeutic results (in four of eleven cases) with nontitrated antileukocytic serum in leukemias seem to indicate that properly prepared and titrated antileukocytic serums may offer an effective remissive method for the treatment in leukemias.

AGRANULOCYTOSIS AND HYPOGRANULOCYTOSIS*

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We have applied the term agranulocytosis to the condition in which there is complete or almost complete absence of granular leukocytes, accompanied by leukopenia and relative increase but, in most instances, absolute decrease of lymphocytes. The term hypogranulocytosis, which was first suggested by Weiss,[1] we have applied to that condition in which there is less marked reduction in the number of granular leukocytes and well marked leukopenia, without absolute increase in the number of lymphocytes but with relative lymphocytosis. These terms are not applied to such distinct clinical entities as acute aplastic anemia, acute leukemia with normal or reduced total leukocyte count and agranulocytosis produced by known poisons such as benzene and roentgen rays; for the purposes of this discussion, however, they are applied to the peculiar blood picture associated with various types of infection.

Brown [2] seems to have been the first to report a case of pharyngeal ulceration with extreme leukopenia, although this fact was overlooked until recently.

Türk,[3] in 1907, reported a case of sepsis with complete disappearance of the granular leukocytes, in which at necropsy there was absence of myeloid elements in the bone marrow.

Schultz,[4] in 1922, reported marked reduction in the number of leukocytes and complete or almost complete disappearance of polymorphonuclears in four cases of necrotic ulcerative infection of the

* Submitted for publication, March 31, 1931.
* From the Mayo Clinic and the Mayo Foundation.

1. Weiss, Julius: Ueber die gegenseitigen Beziehungen zwischen Schulzschem Symptomenkomplex (Mucositis necroticans agranulocytica) akuter Leukämie und septischem Infekt, Wien. Arch. f. inn. Med. **14**:303 (May) 1927.

2. Brown, P. K.: A Fatal Case of Acute Primary Infectious Pharyngitis, with Extreme Leukopenia, Am. Med. **3**:649 (April) 1902.

3. Türk, Wilhelm: Septische Erkrankungen bei Verkümmerung des Granulozytensystems, Wien. klin. Wchnschr. **20**:157, 1907.

4. Schultz, W.: Ueber eigenartige Halserkrankungen, Deutsche med. Wchnschr. **48**:1495, 1922.

throat to which he applied the term agranulocytosis. Friedemann,[5] in 1923, reported similar cases which he called agranulocytic angina. Reviews of the literature have been made by Weiss,[1] Kastlin[6] and Aubertin and Lévy.[7]

There has been much discussion in the literature as to the existence of a distinct clinical entity, agranulocytic angina. Schultz[4] felt that the clinical syndrome to which he applied the term agranulocytosis was a distinct clinical entity, but so many cases have been reported presenting a somewhat dissimilar clinical picture, in which the outstanding feature was agranulocytosis, that the tendency at the present time is to consider the agranulocytosis as a symptom, rather than as a distinct clinical entity.

It is known definitely that certain chemicals, particularly benzene, depress the function of the bone marrow in various degrees, and the effects of thorium X, radium and roentgen rays are equally well known. The lymphocytes are usually affected by irradiation more easily than are the granulocytes, but an overdose of irradiation by any of these means will produce marked reduction of granulocytes and an aplastic effect on the bone marrow.

Farley[8] reviewed from the literature thirty-nine cases in which the function of the bone marrow was depressed following the use of various preparations of arsphenamine. The symptoms varied from those of purpura hemorrhagica to those of severe aplastic anemia and agranulocytosis, depending on whether the principal effect was on the leukopoietic or erythropoietic tissues, on the megacaryocytes or on all of these combined.

Agranulocytosis or hypogranulocytosis conceivably may occur as a result of defective formation of granulocytes, from their abnormal destruction or from their improper distribution or delivery into the circulation. The consensus in the literature is that agranulocytosis usually is due to defective myeloblastic function of the bone marrow, and this is our opinion; but there is some evidence that there is also improper distribution or abnormal destruction. Case 2 is somewhat suggestive of this in the fact that three hours after transfusion the leukocyte count was 300 less than before transfusion. Ordinarily from 500 to 1,000

5. Friedemann, Ulrich: Ueber Angina agranulocytotica, Med. Klin. **19**:1357 (Oct. 14) 1923.

6. Kastlin, G. J.: Agranulocytic Angina, Am. J. M. Sc. **173**:799 (June) 1927.

7. Aubertin, C., and Lévy, Robert: L'agranulocytose et les syndromes agranulocytaires, Arch. d. mal. du coeur **21**:369 (June) 1928; abstr., Medécine **9**:469 (March) 1928.

8. Farley, D. L.: Depressed Bone-Marrow Function from the Arsphenamins (Including a Type of So-Called Agranulocytosis), Am. J. M. Sc. **179**:214 (Feb.) 1930.

leukocytes for each cubic millimeter of blood should have been added by the transfusion, depending on the leukocyte count of the donor and the blood volume of the patient. This is a single observation and does not prove anything. It may be assumed, although it is not known, that the donor had a normal leukocyte count of from 5,000 to 10,000 cells in each cubic millimeter of blood; it is known that the leukocytes numbered 6,100 at the last examination before the transfusion.

We report fourteen cases[8a] in which the blood picture was that of agranulocytosis or hypogranulocytosis. Twelve of the patients came under our observation at the Mayo Clinic in the last three years; one was seen by one of us (Dr. Conner) in consultation with Dr. Gilbert J. Thomas of Minneapolis (case 4), and regarding one case there was a telephone consultation with Dr. J. L. Kestel of Waterloo, Iowa, who was acting as consultant with Dr. E. E. Magee of the same city (case 7).

REPORT OF CASES

CASE 1.—A woman physician, aged 58, had been a patient at the Mayo Clinic three times in previous years. On March 31, 1928, she came to the clinic stating that in the autumn of 1927 she had had trouble somewhat similar to the present complaint, and had been under the care of her home physician. The nodes on both sides of the neck had been enlarged, and her physician had made a diagnosis of pneumonia; she had been in bed for seven weeks; the nodes gradually had receded to normal size and had been of normal size since. Examinations of the blood had not been made. Ten days prior to her admission at the clinic the nodes in the right side of the neck had been swollen and tender; there had been sore throat and dysphagia, malaise, aching in the muscles and joints, loss of appetite and headache, but no chills, cough or thoracic pain. The highest temperature had been 102.8 F.

At examination, the throat was very red, edematous and swollen; there were enlarged, hard nodes on both sides of the neck; the spleen was not palpable, and there were no palpable nodes elsewhere. On April 3 there was pain in the region of the left breast, and a marked pleuritic rub was found; later bronchopneumonia occurred. On April 6, an abscess developed on the left arm, and later required incision. The temperature varied from 101.5 to 104 F., but gradually receded and was normal on the twelfth day. Cultures from the throat revealed streptococci of the viridans and of the hemolytic types, and staphylococci, but no *Corynebacterium diphtheriae*. Two blood cultures gave negative results. Urinalysis disclosed albumin graded 1 and 2, hyaline casts graded 1 and 2, granular casts graded from 1 to 4, an occasional erythrocyte and pus graded from 1 to 3. The diagnosis was agranulocytic angina and bronchopneumonia. The patient returned home after four weeks and made an uneventful recovery.

The patient was readmitted on June 24, 1928. She had made good improvement and had gained 15 pounds (6.8 Kg.). A week before this admission, she had obtained, on her own initiative, roentgen treatment for the nodes of the neck which had not reached normal size. Leukocytes had numbered 2,800 prior to the roentgen treatment, and 1,750, the day after. The day before admission there had been

8a. Since this paper was written six additional cases of agranulocytosis have been observed at the Mayo Clinic.

malaise, nausea, vomiting, a temperature of 102 F. and tenderness and swelling in the cervical and left inguinal nodes. On admission, leukocytes numbered 1,500, 98.5 per cent of which were lymphocytes and 1.5 per cent, neutrophils. Further results of blood counts are given in the table. There was a questionable pleuritic rub on the left, and some enlargement of the upper cervical nodes and the nodes in both inguinal regions. Later, a node in the right groin underwent suppuration. Still later, an inflammatory condition of the vulva developed. Blood cultures, on June 30 and July 2, contained *Klebsiella pneumoniae* (Friedländer's bacillus). On July 2 and 3, six injections of 10 cc. of leukocytic extract were made subcutaneously. The temperature varied from 101 to 104 F. until the day of death, when it was 105.5 F. by axilla, and the concentration of urea rose to 92 mg. in each 100 cc. of blood. Before death there was evidence of bronchopneumonia and a suggestion of ileus. Permission to perform necropsy was refused.

Comment.—This is a case of undoubted agranulocytic angina (Schultz's syndrome). Although the nature of the attack six months prior to admission is somewhat doubtful, as incomplete observation was made, it seems likely that it was of the same kind as the last two. It is interesting that there was leukopenia five weeks before the patient came under our observation, but that several former blood counts were normal, indicating that the bone marrow was formerly active. The increase in leukocytes and granulocytes soon after development of pneumonia and the abscess on the arm suggest the possibility of using an acute infection therapeutically, for ordinarily such infections are accompanied by leukocytosis. The possible effect of the roentgen treatment of the nodes in bringing on the last attack must be kept in mind. There was no noticeable effect from the use of leukocytic extract. The occurrence of *Klebsiella pneumoniae* in the blood may have been of little significance.

Case 2.—A woman, aged 37, a hospital nurse, came to the Mayo Clinic on Nov. 15, 1928. She had first visited the clinic in 1908, for appendectomy, and five times subsequently for keratitis, cough and persistent general debility which never could be attributed to any particular disease. She had been in the hospital on several occasions. There had been mild secondary anemia on several examinations. One week prior to admission the left middle finger had become infected, painful and swollen. Four or five days later, fever, headache, weakness and nausea had occurred. Examination of the blood had been made two weeks before admission, and the number of leukocytes in each cubic millimeter of blood had been found to be 2,000.

At examination, the pharynx and mouth were normal; there were swelling and tenderness in both submaxillary regions, induration of the submental region, some enlargement of the cervical nodes, but none of the axillary and epitrochlear nodes, a slight residual infection of the finger, a few ecchymotic and purpuric areas on the back, a few râles in the base of the right lung and a doubtfully palpable spleen. There were 3,200 leukocytes in each cubic millimeter of blood, 100 per cent of which were lymphocytes. Further counts are given in the table. Three days after admission there developed a sore throat, with no distinct ulceration, and an impetiginous eruption on the face. The patient rapidly became worse, and three days before death an inflamed, indurated area developed on one thigh. A

transfusion of blood was given on November 22. The leukocytes, before transfusion, numbered 500, and three hours after transfusion, 200 in each cubic millimeter of blood. The patient died on November 24 from terminal bronchopneumonia. The diagnosis was agranulocytic sepsis without angina.

At postmortem examination numerous herpetic lesions were found over the face and eyelids. There were some petechiae over the sclerae. The submaxillary glands on both sides appeared to be somewhat swollen. Over the mesial surface of the left thigh there was an indurated area 10 cm. in diameter, of purplish color. On section, pus was not exuded. There were numerous petechial hemorrhages over the epicardium. The myocardium was somewhat flabby. The lower lobe of the left lung was of a dark purplish color, its consistency was markedly increased and crepitus could not be felt. The color of the surface after section was diffusely purple, and numerous discrete hemorrhagic areas were seen. There were an excess of bloody fluid and complete absence of air. The lower lobe of the right lung revealed similar nodular, hemorrhagic areas. The mucosa of the pyloric region of the stomach was injected, and there was present what appeared to be a recent ulcer, 1 cm. in diameter. In the cecum there were thirty or forty ulcers, some of which were covered by a pseudomembranous exudate. The bone marrow of the diaphysis of the femur was yellow and fatty. The bone marrow was absent from most of the ribs, but some ribs contained red marrow.

The bone marrow from the shaft of the femur was composed essentially of adipose tissue and a prominent reticulum. In the meshes of the reticulum the cells were sparse; they were normal erythrocytes, an occasional nucleated erythrocyte and what appeared to be immature leukocytes. No definite myelocytes or mature polymorphonuclear cells were observed.

The pleura over the lower lobe of the left lung was somewhat thickened. There was marked congestion of the blood vessels. Many of the alveoli were filled with serum and erythrocytes. Some alveoli contained small amounts of fibrin, but there was a striking lack of much cellular infiltration. The cells that were present within alveoli were either lymphocytes or endothelial leukocytes filled with phagocytosed blood pigment. Essentially the same changes were observed in the lower lobe of the right lung, except that there a feeble attempt at organization of the exudate by proliferation of fibroblasts apparently had been made. A section of one of the submaxillary glands revealed edema and necrosis of tissue, particularly in the stroma of the gland and in the connective tissue capsule (fig. 1). There was a diffuse, and, in places, a dense cellular exudate composed almost entirely of small lymphocytes and endothelial leukocytes. Polymorphonuclear leukocytes were practically entirely absent. The stomach revealed a number of small ulcers, the bases of which were filled with necrotic tissue and clumps of bacteria (fig. 2). The necrosis extended for a variable depth, in places penetrating the muscularis mucosae. In the colon there were numerous small ulcers; there was no inflammatory cellular reaction about the regions of necrosis. The skin of the left thigh revealed marked edema of the corium with vascular congestion and small hemorrhages. About some of the vessels there was cellular infiltration composed entirely of small lymphocytes.

The anatomic diagnosis was agranulocytic sepsis; bilateral bronchopneumonia; submaxillary adenitis; multiple visceral hemorrhages; multiple ulcers of the stomach and colon; cellulitis of the thigh and neck, and atrophy of the bone marrow.

Comment.—This was a typical case of agranulocytic angina (Schultz's syndrome) except that there was no distinct ulceration of

the oropharyngeal mucous membrane, but there were ulcerative cutaneous lesions of the face and thigh. The extreme reduction of leukocytes to 100 in each cubic millimeter of blood before death is noteworthy. A heretofore unreported phenomenon is the rapid disappearance of transfused leukocytes and of the patient's own leukocytes, so that from 500 just before transfusion they were reduced to 200 three hours after transfusion. Note should be made that in this case there were 2,000 leukocytes a week before the patient became ill.

CASE 3.—A woman, aged 54, a housewife, came to the Mayo Clinic on May 12, 1930. Her history, before her present illness, was essentially unimportant. In

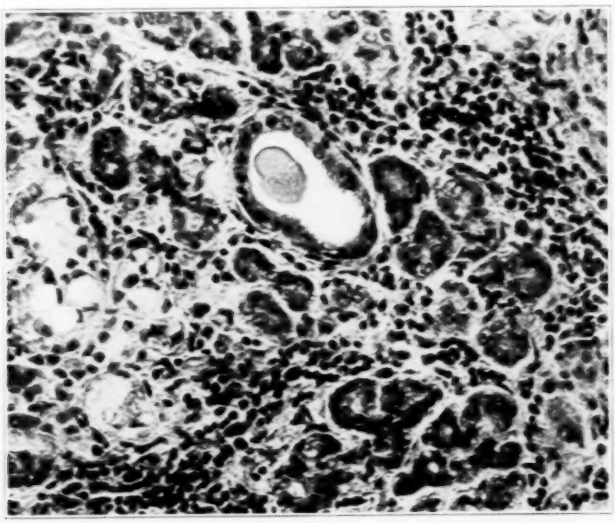

Fig. 1.—Lymphocytic infiltration mainly in edematous stroma of submaxillary gland and some degeneration of glandular epithelium in a case of agranulocytosis. There is complete absence of polymorphonuclear leukocytic infiltration. Hematoxylin and eosin; × 350.

July, 1929, a sore throat developed. Two weeks later the leukocytes numbered 1,000 in each cubic millimeter of blood; the proportion of polymorphonuclears was 11 per cent. A diagnosis of agranulocytic angina was made by her physicians at home, and the case was reported in detail by Dr. G. Parker,[9] who saw her in consultation. She was referred to us by Dr. H. W. Brink of Delavan, Ill., for further investigation. At the time of admission she was still easily fatigued and occasionally had a sore throat.

At examination, small hemorrhoids and fibrous tonsils with sealed crypts were found. Examination otherwise gave negative results. Blood counts are given in

9. Parker, George: Agranulocytosis with Case Report, Illinois M. J. **58**:440 (Dec.) 1930.

the table. There were five teeth with periapical infection and two impacted wisdom teeth. A diagnosis of agranulocytic angina was made from the history, although evidence of this disease was not present at the time the patient was under our care. Tonsillectomy was performed without promise of benefit. It was suggested that, if no improvement occurred in a few months, the infected teeth be extracted. The patient's general condition was good at the last report.

Comment.—This seems to have been a mild case of agranulocytic angina (Schultz's syndrome), although the neutrophils were never below 11 per cent of the total of 1,000 leukocytes (hypogranulocytosis). Neither the patient nor her family knew of her having suffered, at any time, from ulceration of the throat. What effect the various methods

Fig. 2.—Ulceration in stomach and necrosis of tissue, and almost complete absence of cellular infiltration in a case of agranulocytosis. Hemotoxylin and eosin; × 20.

of treatment had is conjectural. The leukopenia had persisted, to the time of the last report, in spite of removal of evidently infected tonsils. The occurrence of another attack at a later date is to be feared.

CASE 4.—A business man, aged 62, entered the Eitel Hospital at Minneapolis on July 22, 1926. In 1916 or 1917 he had had gross hematuria, and in 1919 microscopic hematuria; cystoscopic examination had given negative results. Three weeks before entry he had had gross hematuria, frequency and urgency; general examination gave negative results, and cystoscopic examination disclosed papilloma of the bladder. On July 24, suprapubic cystostomy and cauterization of the tumor were performed by Dr. Gilbert J. Thomas. The day after the operation, fever developed and continued intermittently until sixty-three days after operation. About thirty-three days after the operation sloughing of the operative wound

occurred, and the wound opened. About twenty-four days after the operation, marked ulceration of the mouth and oropharynx developed and included the tonsils and their pillars. The patient's general condition was grave. Examination of the blood on August 6 disclosed that there were 10,700 leukocytes in each cubic millimeter of blood. On August 22, about the time of the beginning of ulceration, the leukocytes numbered 3,600 and on August 23, 3,100; on August 24, the percentage of polymorphonuclears was 4 and of lymphocytes, 96. Further blood counts are given in the table. For several days it was impossible to find a polymorphonuclear, but on August 26 they again appeared, and in two days their percentage again was normal. From September 13 to September 22, the number of leukocytes again was slightly diminished at times and the percentage of polymorphonuclears varied from 47 to 56, but there was no recurrence of symptoms. The patient made a good recovery, and recurrence has not taken place.

Comment.—This case fits well into the group of cases of agranulocytic angina (Schultz's syndrome), although the condition appeared after operation. The recovery after complete disappearance of polymorphonuclears for four days is unusual. Whether the operation or the anesthetic had anything to do with the production of the agranulocytosis is conjectural.

Case 5.—A housewife, aged 51, came to the Mayo Clinic on May 19, 1930, and because of abdominal pain and a roentgenologic diagnosis of diaphragmatic hernia she underwent operation on June 21, 1930. Convalescence was uneventful. Leukocytes had numbered 8,700 in each cubic millimeter of blood, May 19, 1930; 9,600, June 25, and 8,500, July 2. The patient returned to the clinic on December 23. She had been well until one week previously, when the following conditions had developed: headache; abdominal soreness with flatulence, and sore mouth and throat which had been different from any previous attack, and which had been associated with aphthous ulcers. She evidently had had fever for several days before admission, and the sore throat had become worse.

At examination, the temperature was 101.8 F., but except for this, aphthous stomatitis was the only significant condition found. Leukocytes numbered 2,500 and erythrocytes 4,260,000 in each cubic millimeter of blood. The concentration of hemoglobin was 13.1 Gm. in each 100 cc. of blood (normal concentration, from 15 to 17 Gm.). On the following day, December 24, leukocytes numbered 1,400; the percentage of neutrophils was 2; of lymphocytes, 84, and of monocytes, 14. Platelets numbered 118,000 in each cubic millimeter of blood. The patient was hospitalized December 26, at which time her temperature was 100 F., and leukocytes numbered 6,000, of which 51 per cent were polymorphonuclears. December 27, the temperature was normal; leukocytes numbered 3,400, of which 45 per cent were neutrophils. A blood culture was negative, and a culture made from a throat swab disclosed green-producing streptococci. There was a gradual rise in the number of leukocytes and in the percentage of neutrophils, until, at the time of dismissal of the patient, January 3, leukocytes numbered 7,600, and the percentage of neutrophils was 67; of monocytes, 3; of lymphocytes, 30, and of reticulated cells, 1.4. Platelets numbered 136,000 in each cubic millimeter of blood. The smears appeared to be practically normal. The stomatitis had improved markedly, and the patient's general strength was much increased. A diagnosis of mild agranulocytic angina, with spontaneous recovery, was made. No treatment of any kind was administered.

The patient went home on Jan. 3, 1931, and returned on January 15. She had been feeling well. On January 13, leukocytes had numbered 3,560, and on January 14, 2,930. The patient returned at her physician's advice, feeling perfectly well. She had not had sore throat or sores elsewhere, and only a few ulcers of aphthous stomatitis. At examination ulcers were not seen. On January 15, the concentration of hemoglobin was 75 per cent, and erythrocytes numbered 4,840,000 and leukocytes, 1,400, in each cubic millimeter of blood. The percentage of lymphocytes was 76.5; of large mononuclears, 11; of neutrophils, 11, and of basophils, 1. Platelets numbered 172,000. No enlargement of glands or nodes was found, and the spleen was not palpable. The patient was given roentgen treatment over the upper end of each femur and humerus and in both cubital spaces, on the basis of results obtained by Friedemann and Elkeles.[10] A blood culture was again negative, and a culture made from throat swabs contained green-producing streptococci, gram-positive bacilli and staphylococci. Four days later a moderate number of small ulcers was present; they appeared much like those of aphthous stomatitis. At the time the ulcers appeared, leukocytes numbered 5,300; the percentage of lymphocytes was 67; of large mononuclears, 13, and of neutrophils, 30. The ulceration gradually spread to the anterior pillars and involved the tonsillar region. There was slight swelling in the cervical region and some tenderness. The ulcers gradually improved, and by January 26 were almost completely healed. The leukocyte count continued to be high, compared to what it had been. No cause could be found for this increase in the number of leukocytes except possibly the stimulation of the bone marrow by the roentgen rays. The patient has been under observation until the time of the writing of this report and has not shown evidence of recurrence of the condition.

Comment.—Apparently this is a case of agranulocytic angina (Schultz's syndrome); the second attack was especially characteristic. The ulceration, although definite, was not marked. The rapid recovery from the first attack, with the early relapse, and recovery from the second attack are noteworthy. It may be possible that the recovery can be attributed to the roentgen treatment, given according to the method of Friedemann, with the use of 1/20 erythema dose. The increase in the number of leukocytes following its use is worthy of note.

Case 6.—A housewife, aged 48, first came to the Mayo Clinic on Aug. 19, 1921; she returned seven times in the ensuing nine years. Leukocytes in 1921 and 1922 were normal in number.

Subtotal abdominal hysterectomy was performed on Nov. 4, 1930, for fibromyomas. Recovery was uneventful for ten days. Then slight, apparently superficial, pain developed in the left side of the thorax, and slight tenderness in the pelvis. General examination of the thorax gave negative results. Evidence of phlebitis was not found. Seventeen days after operation slight pain developed on the inner surface of the right thigh, which gradually became worse and involved the whole thigh. There was deep-seated tenderness but no edema. A diagnosis of phlebitis was made, the leg was elevated and heat was applied. Leukocytes, ten days after operation, numbered 18,800 in each cubic millimeter of blood. On

10. Friedemann, U., and Elkeles, A.: Die Roentgenbehandlung der Agranulozytose, Deutsche med. Wchnschr. **56**:947 (June 6) 1930.

December 11, thirty-seven days after operation, sore throat and a temperature of 103 F. developed. On December 12, leukocytes numbered 4,600. On December 15, the number of leukocytes had fallen to 2,000, of which none was a polymorphonuclear; erythrocytes numbered 4,580,000, and the concentration of hemoglobin was 65 per cent. Further blood counts are given in the table. The temperature continued high until the death of the patient, December 21. The patient was extremely weak, but her appearance was not that of extreme illness. Two blood cultures, a Widal test and an agglutination test for undulant fever gave negative results. Typhoid bacilli were not found in the stools. There was a moderate amount of albumin in the urine, and the roentgenogram of the thorax did not disclose any abnormality. The patient's course was rapidly downward. Large doses of yellow bone marrow were given without effect, and one transfusion caused a severe reaction. Roentgen treatment was given over both shoulder girdles and over the cervical region, without effect. Death occurred on December 21. Records of necropsy are not available.

Comment.—This case should probably be classified with those of agranulocytic angina, although soreness of the throat was not marked and there was no definite ulceration. Agranulocytosis was complete for five days before death. Leukocytes numbered 18,800 in each cubic millimeter of blood a month before the appearance of the agranulocytosis. This suggests either that the depression of the bone marrow resulting in the agranulocytosis was a sequel of the infection, which was accompanied by leukocytosis, that it was the result of the infection causing the sore throat, or that it was the result of some unknown factor that depressed the bone marrow in the interval between the two infections. A rapidly fatal outcome a short time after a patient has seemed relatively well has been noticed by others.

CASE 7.—A woman, aged 30, was admitted to the hospital in Waterloo, Iowa, on March 10, 1929. She had been ill one week with a condition of the throat that was thought to be Vincent's angina. There was considerable ulceration in the tonsillar region and adjacent structures. Prostration was fairly marked; the temperature was 102 F.; the pulse rate was 120 beats each minute, and respirations were 20 each minute. Examination did not reveal any abnormality except ulceration and redness in the throat. The concentration of hemoglobin was 85 per cent; erythrocytes numbered 4,250,000 and leukocytes 6,700 in each cubic millimeter of blood. The percentage of lymphocytes was 90; of large mononuclears, 9, and of polymorphonuclears, 1. Smears from the throat contained Vincent's organisms. The following day the patient was irrational; her temperature was 104.4 F.; leukocytes numbered 6,100, and polymorphonuclears were absent. Further blood counts are given in the table. The next day the gums and mouth were painful, swollen and bleeding, and the swelling extended to the sides of the neck. The patient gradually grew worse; two transfusions of blood were given, and diphtheria antitoxin was administered. Nine days after admission the patient seemed better; the number of leukocytes had risen to 3,950, and the proportion of polymorphonuclears was 76 per cent. Eleven days after admission the patient's condition suddenly became worse, and she died soon thereafter. At the time of her death the leukocytes numbered 7,600, and the proportion of polymorphonuclears was 86 per cent.

Comment.—From the description, this case seems to have been one of agranulocytic angina. The rather sudden death in the presence of normal values for the total number of leukocytes and a high percentage of granulocytes, even though these were somewhat immature, seems likely to have occurred as a result of some complication.

CASE 8.—A girl, aged 4 years, whose family history was unimportant, was brought to the Mayo Clinic on Dec. 29, 1929. Her health had been good until the onset of the illness for which she was brought to the clinic. In July she had become pale and listless, and in the six weeks preceding our examination, weakness and anorexia had been marked. There had been some vomiting, dark stools, mild abdominal pain and possibly fever.

At examination, pallor was marked; there was a systolic murmur over the whole precordium; the pulse was moderately accelerated; the spleen and liver were not palpable. The concentration of hemoglobin was 10 per cent; erythrocytes numbered 860,000 and leukocytes 4,000 in each cubic millimeter of blood. The proportion of lymphocytes was 76 per cent, and of neutrophils, 18 per cent. Additional blood counts are given in the table. The Wassermann reaction of the blood, examination of stools and roentgenologic examination of the stomach, colon and thorax gave negative results. Emergency transfusions of blood were given at entry, and many were given later. The second day the tonsils were swollen and red, there was some exudate on the surface and oral fetor was noted. The cervical and left axillary nodes were moderately enlarged, and by rectum a small, smooth mass to the left of the median line was felt. Cultures made from the tonsils did not contain *Corynebacterium diphtheriae,* but many fusiform organisms and spirilla were found. Two blood cultures gave negative results, but *Escherichia coli* was found in the urine repeatedly. On Jan. 13, 1930, a small dose of radium was applied to the left submaxillary region. The cervical adenopathy was greatly reduced, and the throat and mouth were practically clear. Several furuncles appeared on the neck, and the symptoms referable to the throat recurred. The temperature continued to be elevated, and bilateral otitis media developed. The inguinal adenitis and the furunculosis in the sternal region progressed to formation of abscess. Cellulitis developed in the region of the left side of the jaw. A cervical abscess was drained surgically. The inguinal adenitis became suppurative, and the abscesses were drained. On February 19, acute tonsillitis with cervical adenitis again developed, and was more marked on the right. On February 21, there was a membranous exudate involving a large portion of both tonsils, and two days later, superficial ulceration. A culture contained Vincent's organisms. Leukocytes numbered 1,000 in each cubic millimeter of blood, of which the percentage of lymphocytes was 84, and of polymorphonuclears, 14. The smears did not, at any time, contain immature leukocytes suggestive of acute leukemia. The ulceration spread widely. Jaundice and cyanosis rapidly developed and continued until the death of the patient, Feb. 24, 1930. The patient's temperature varied from 99 to 106 F. while she was in the hospital. A diagnosis of agranulocytic angina (hypogranulocytosis) seemed more justified than others. Fetal liver, 1 drachm (3.91 Gm.) daily, was given during most of the period of hospitalization.

At necropsy, the distal third of the appendix was reddish purple; it was coiled, moderately distended and covered with a yellowish, fibrinous exudate. A number of ileocecal lymph nodes were enlarged.

There were numerous petechial hemorrhages scattered diffusely in the subepicardial tissue. A minute vegetation, apparently of recent origin, was present

on one of the cusps of the mitral valve. There were numerous subpleural petechial hemorrhages scattered over the surfaces of both lungs, and over the anterior surface of the right upper lobe was an area of subpleural hemorrhage, measuring 1 cm. in diameter.

The superior surface of the tip of the tongue was discolored, due to hemorrhage, and there was an area of ulceration, measuring 4 mm. in diameter, which was covered with yellowish exudate. A similar exudate also covered the base of the tongue, about the circumvallate papillae. The faucial tonsils were moderately enlarged and deeply hemorrhagic. Over the epithelial surface of the tonsils, dipping into their crypts, was a yellowish, fibrinous exudate. In the crypts the exudate was intimately attached, whereas on the surface of the mucous membrane it could be easily peeled off. There was considerable necrosis of the superficial layers of tissue, and in places the necrosis extended deeply into the tonsils. The pharynx was deeply hemorrhagic and was covered by an exudate similar to that present on the tonsils; there were beginning sloughing and ulceration of the mucosa. The epiglottis was mildly congested. The rima glottidis was edematous, the edema effecting nearly complete closure. The upper part of the larynx, down to and including the vocal cords, was also markedly edematous and congested, and was covered with yellowish exudate such as was present in the pharynx. The mucosa of the vocal cords presented the appearance of superficial ulceration.

The gastro-intestinal tract appeared normal except for the presence of three punched-out ulcers 0.5 cm. in diameter in the terminal portion of the ileum.

The mucous membrane of the urinary bladder was edematous, and there were numerous small hemorrhagic areas in the submucosa. The thymus appeared to have undergone complete involution. The diaphyseal marrow of the femur presented a reddish appearance.

Smears from the tonsils, made after death, revealed *Neisseria catarrhalis* (*Micrococcus catarrhalis*), streptococci, *Borrelia vincenti* and *Fusiformis dentium* (Vincent's organisms). Cultures made under the same circumstances, from the contents of the appendix, contained streptococci of a hemolyzing strain, in long chains, and *Escherichia coli*. Cultures from the serosa of the appendix contained *Staphylococcus aureus* in pure culture, and those from the bone marrow of the right femur contained a hemolyzing strain of streptococci in long chains.

Sections of the tonsils and of the pharynx presented extensive regions of ulceration and necrosis (figs. 3, 4, 5 and 6). The necrotic tissue remained attached to the base of the ulcer; it was matted together by dense masses of fibrin in which there were clumps of deeply blue-staining material of varying size, probably bacteria. There was extensive thrombosis of the smaller blood vessels. In the pharynx the ulcer extended for a variable depth, in places reaching the muscular layer. In the tonsils the necrosis was most marked about the crypts, and extended as deeply as the fibrous capsule, even traversing it in places. The cellular reaction about the necrotic tissue was very feeble and was composed mainly of small and large lymphocytes and scattered endothelial leukocytes. The polymorphonuclear leukocytes were extremely scanty. There were areas of ulceration of the mucosa of the tongue in the midst of an area of widespread necrosis. The epithelium, in places, remained attached to the underlying tissue, although almost all of it was necrotic. The necrosis extended deeply into the muscular tissue, but the necrotic tissue showed no tendency to separate from its base. There was practically no cellular reaction about the region of necrosis. There were edema of the mucous membrane of the epiglottis and small, focal areas of necrosis, surrounded by infiltration of small and large lymphocytes and immature fibroblasts.

Fig. 3.—Ulceration and diffuse necrosis in the tonsil, with the presence of clumps of bacteria, in a case of agranulocytic angina. Hematoxylin and eosin; × 20.

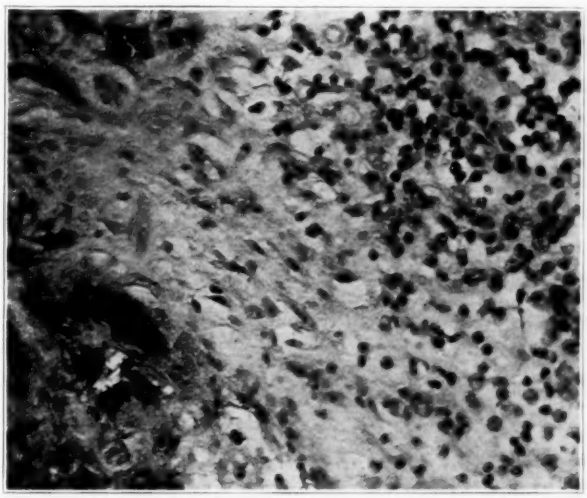

Fig. 4.—Base of necrotic region in tonsil shown in figure 3, to indicate the sparsity of the cellular infiltration and the marked predominence of lymphocytes. Hematoxylin and eosin; × 350.

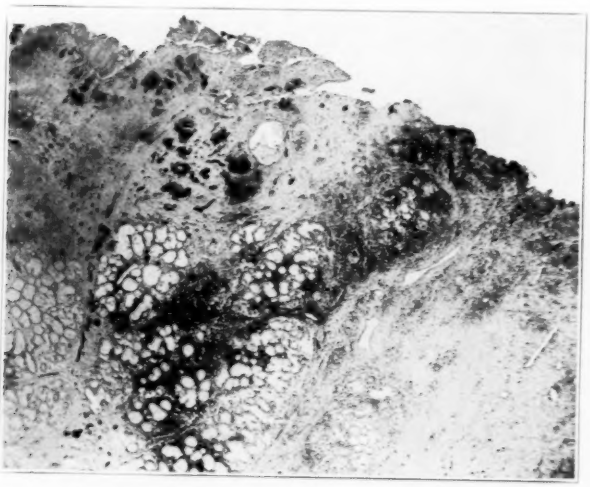

Fig. 5.—Ulceration of pharyngeal mucosa and necrosis of pharyngeal wall with the presence of clumps of bacteria in a case of agranulocytosis. Hematoxylin and eosin; × 20.

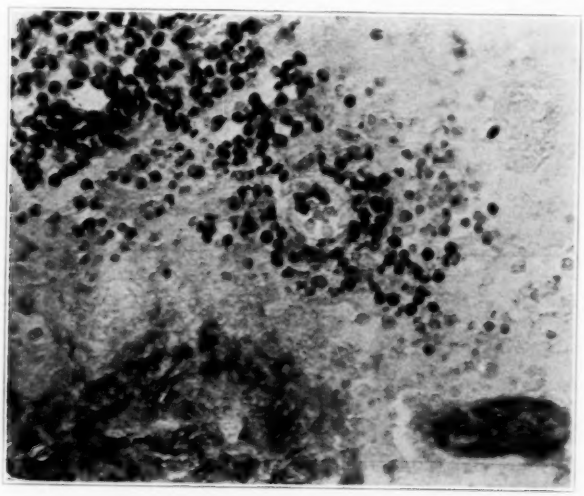

Fig. 6.—Base of pharyngeal ulcer, showing very sparse cellular infiltration composed almost entirely of lymphocytes and an occasional polymorphonuclear leukocyte. Hematoxylin and eosin; × 350.

In the bone marrow of the diaphysis of the femur were a small number of cells, and the prominence of the reticulum was increased (fig. 7). There were many megaloblasts and occasional normoblasts. Cells of the myelocyte series were hardly to be seen, although here and there an occasional eosinophilic or neutrophilic polymorphonuclear cell could be found. There were, in addition, some large and some small lymphocytes, and an occasional megakaryocyte. Macrophage cells containing phagocytosed blood pigment were numerous. The hepatic parenchyma presented diffuse granular degeneration and a focal region of necrosis about which there was no cellular reaction. There was a deep ulcer in the ileum, filled with necrotic tissue, fibrin and large clumps of bacteria, but there was no

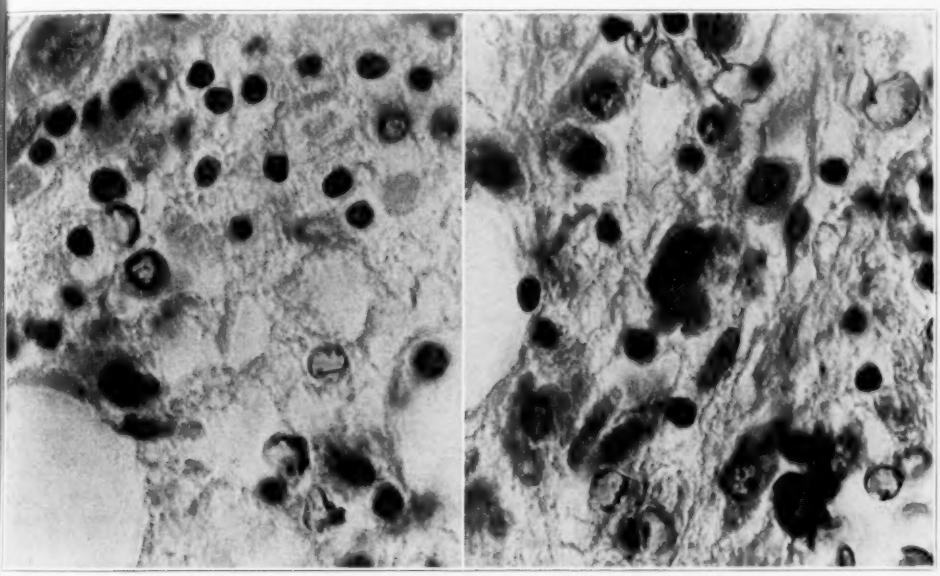

Fig. 7.—Bone marrow in a case of agranulocytic angina showing prominence of cells of reticulum, small and large lymphocytes and endothelial leukocytes (macrophage cells). Granular cells of myeloid series are absent. Hematoxlin and eosin; × 900.

cellular reaction about this region of ulceration (fig. 8). The appendix presented large regions of necrosis, containing large clumps of bacteria; there was some infiltration with lymphocytes about these regions, but there were practically no polymorphonuclear cells. The microscopic study of the other tissues revealed nothing notable pathologically.

The anatomic diagnosis was agranulocytic angina; acute laryngitis with edema of the glottis; acute appendicitis, superficial ulceration of the terminal portion of the ileum; focal necrosis of the liver; petechial hemorrhages of the pleurae, heart and urinary bladder, and involution of the thymus.

Comment.—The notable features in this case were the marked anemia and the hypogranulocytosis. The anemia was not of the aplastic type, as shown by the normal or increased percentage of reticulocytes and the fairly normal platelet level. Otherwise, the case would correspond fairly well with agranulocytic angina. Administration of fetal liver apparently had no effect. The presence in the bone marrow of streptococci of a hemolyzing strain, found at necropsy, is of interest.

Case 9.—A housewife, aged 47, came to the Mayo Clinic on July 25, 1929. The family history was unimportant. She had had influenza, scarlet fever and diphtheria. In February, 1929, cold in the head and "grip" had developed, and she had been in

Fig. 8.—Ulceration and necrosis in superficial layers of the ileum, and absence of cellular infiltration in a case of agranulocytic angina. Hematoxylin and eosin; × 100.

bed one week. She had continued to be weak; some soreness of the throat had developed again, and she had been in bed nine weeks. Beginning in April she had had some fever, had continued to be weak, had received some treatment of the accessory nasal sinuses and had been given a diet high in calories. Leukocytes had numbered 2,750 in each cubic millimeter of blood, of which 83 per cent were lymphocytes and 17 per cent polymorphonuclears. The Widal test and blood cultures had given negative results. The patient had become progressively weaker, had vomited in the week just before her admission to the hospital and had had a slight cough and a slightly sore throat.

At examination the temperature was 99.6 F., and the pharynx was red. General examination otherwise gave negative results. The concentration of hemoglobin

was 61 per cent; erythrocytes numbered 4,510,000 and leukocytes 2,300. The percentage of lymphocytes was 76, and of neutrophils, 11. There was only an occasional polymorphonuclear in the blood smears. The Wassermann reaction of the blood was negative, and achlorhydria was present. Roentgenograms of the thorax, stomach and duodenum gave negative results. There were six infected teeth, and the tonsils had been cleanly removed. The diagnosis was probable mild agranulocytic angina (hypogranulocytosis). The patient was treated with an extract of yellow bone marrow prepared from long bones. Subsequent blood counts made by her physician at home are given in the table. Her physician reported that she is now in excellent condition.

Comment.—This case probably was one of mild agranulocytic angina (Schultz's syndrome) with hypogranulocytosis only. At one time, examination of the blood smears disclosed almost no polymorphonuclears, although the lowest number recorded was 11 per cent of 2,300 leukocytes (hypogranulocytosis). The apparent recovery with the use of an extract of bone marrow is worthy of note, although its occurrence may be only coincidental.

CASE 10.—A housewife, aged 31, came to the Mayo Clinic on July 21, 1930. She had had good health until March, 1930, when she had had a sore throat, with swelling of the neck and dysphagia, lasting four or five days. The gums had become spongy and sore and had bled somewhat; local treatment had resulted in improvement. She had felt well until May, and the soreness had disappeared. In the two weeks previous to her registration at the clinic she had had a sore throat again and also herpetic lesions on the lips. There had been a small ulcer on the tongue, which had been greatly swollen. There had been soreness of the right cheek and swelling of the right side of the face, which had persisted to the time of her entry. There had been a small blister in the right axilla in the week previous to registration.

At examination there was indurated swelling of the right parotid and submaxillary glands, and of the right side of the neck, and there was some ulceration of the right cheek. There were superficial ulcerations in various portions of the mouth which suggested Vincent's angina, and a smear from the throat contained Borrelia vincenti and Fusiformis dentium. The concentration of hemoglobin was 60 per cent; erythrocytes numbered 3,860,000, and leukocytes 4,500. The percentage of small lymphocytes was 42; of lymphocytes, 20; of transitionals, 15; of neutrophils, 22, and of eosinophils, 1. Additional blood counts are given in the table. The patient was given 3 grains (0.195 Gm.) three times daily of yellow bone marrow extract and 20 grains (1.3 Gm.) three times daily of ferric citrate. There was gradual improvement, the inflammatory mass disappeared and the ulcers healed. The diagnosis was probable agranulocytic angina, and the possibility of recurrence was explained to the patient. She was dismissed August 16 in fair condition.

Comment.—There were apparently two separate attacks. This case is of doubtful diagnosis as the total number of lymphocytes was actually increased at times. Sometimes the blood picture was that of lymphocytosis, at other times that of hypogranulocytosis. Nevertheless, it is somewhat likely that the condition in this case is closely related to agranulocytic angina (Schultz's syndrome), although the low-

est number of polymorphonuclears was 20 per cent of 4,900 leukocytes (hypogranulocytosis). The improvement during the use of bone marrow may or may not be significant.

CASE 11.—A farmer, aged 59, came to the Mayo Clinic on Oct. 2, 1929. The family history was unimportant. For five years he had had flatulence after meals, which had been worse with certain foods. He had had pain in the right upper abdominal quadrant about twice weekly, and a severe attack three weeks previous to his registration at the clinic; fever and weakness had been present since the last attack. No nausea, vomiting or jaundice had been noted, but there had been marked constipation, a small urinary stream, recent anorexia and nocturia graded 1 the previous year. For four years there had been occasional shooting pain in the

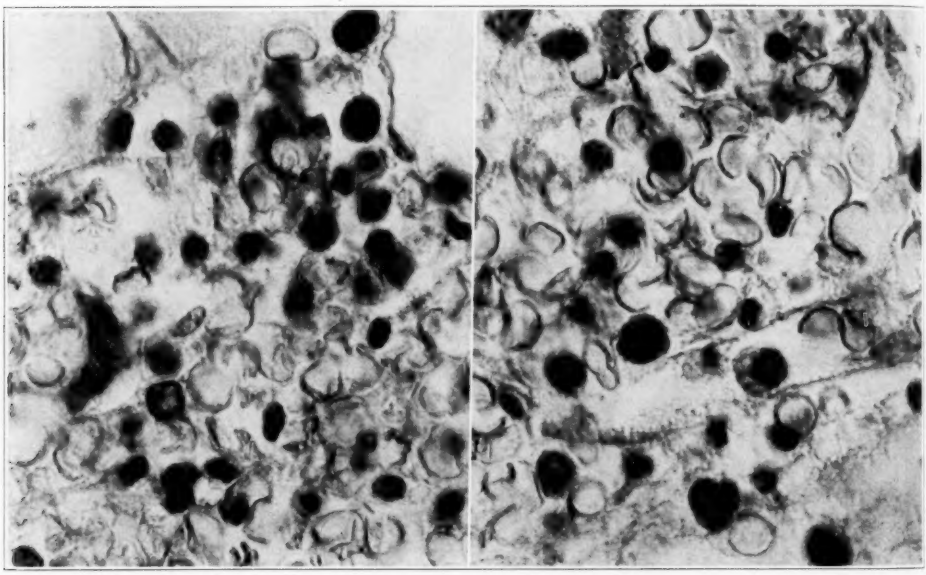

Fig. 9.—Bone marrow in a case of agranulocytosis, showing numerous plasma cells, lymphocytes and occasional normoblasts. There is almost complete absence of granular leukocytes of the myeloid series. Hematoxylin and eosin; × 900.

left flank, radiating to the groin. The patient's complaint was of weakness, jaundice, fever and abdominal pain.

At examination, the pulse rate was 116 beats each minute, the blood pressure was 112 systolic and 80 diastolic, and the temperature was 102 F. Jaundice graded 2 was present. A mass in the left upper abdominal quadrant apparently was the spleen. The liver was approximately normal to percussion, but the edge was not felt. There was no ulceration of the throat. Albuminuria was graded 2 to 3; the urine contained bile, granular casts graded 1 and an occasional pus cell. Tyrosine was found in the urine twice by chemical examination. The return of phenolsulphonphthalein was 30 per cent, and 225 cc. of residual urine was

present. Roentgenograms of the kidneys, ureters and bladder were unsatisfactory, and those of the colon gave negative results; roentgen examination indicated that the gallbladder was not functioning. The Wassermann reaction of the blood was negative. Examination of stools gave negative results for parasites and blood, but the stools contained bile. The concentration of gastric acids was normal. Three blood cultures contained *Salmonella schottmülleri (Bacillus paratyphosus B)*. Agglutination tests for undulant fever, typhoid fever and tularemia gave negative results; agglutination tests with *Salmonella schottmülleri* gave positive results in a dilution of 1:2,560. The fragility test disclosed increase in resistance of erythrocytes. The concentration of urea was 38 mg. in each 100 cc. of blood, and of bilirubin, was variously 4.1, 4.2 and 3.8 mg. in each 100 cc. of serum. The van den Bergh reaction was direct. The concentration of hemoglobin was 35 per cent, and the erythrocytes numbered 1,960,000 in each cubic millimeter of blood. The number of leukocytes in each cubic millimeter of blood was, successively, 2,200, 1,100, 1,100, 1,200, 1,000, 600, 900, 700, 1,200, 1,000, 1,000, 1,100, 1,000, 3,000, 1,800, 400, 1,600, 1,800, 1,500, 800 and 1,600. Further data are given in the table. The percentage of polymorphonuclear leukocytes was 13 on the day of the patient's admission to the clinic; 56, two weeks later; 64, sixteen days after entry, and 20, three days before death. Immature leukocytes were not found, except on one occasion. The diagnosis was *Salmonella schottmülleri* septicemia with agranulocytosis. Six transfusions of 250 cc. of blood were given, and 200 mg. of acriflavine was given intravenously. The patient gradually became worse, went into a typhoid state and died. Before death, generalized impetiginous eruption developed, especially over both buttocks, and a bullous eruption appeared in the mouth, which somewhat simulated the oral lesions of pemphigus.

At necropsy numerous bluish macules, from 1 to 8 mm. in diameter, were found over the skin of the trunk, laterally and posteriorly. There was moderate jaundice.

The epicardial fat was bile-stained, and there were some petechial hemorrhages over the posterior surface of the right ventricle, near the base. The myocardium was somewhat softened, and in the subendocardial tissue there was a faint yellowish mottling, as of fat. There were small, firm, nodular lesions, from 1 to 2 cm. in diameter, scattered through both lungs, the result of consolidation. The spleen weighed 1,225 Gm., was of normal consistency and presented a diminution in the prominence of the lymphoid follicles. The liver weighed 2,565 Gm., but showed no other significant gross pathologic changes. The esophagus revealed, in its lower portion, many superficial erosions. In the cecum, ascending colon and proximal half of the transverse colon, there were many mucosal abscesses, measuring from 1 to 2 mm. in diameter. There was some edema and some hemorrhage involving the mucosa of the bladder. The prostate gland was slightly enlarged. The entire right lobe had been destroyed and was replaced by an abscess. In the left lobe there was an abscess measuring 1 cm. in diameter.

Sections of the bone marrow of the shaft of the femur revealed that the cells were sparse and that there was a moderate increase in the amount of fat (fig. 9). The most striking feature was the presence of numerous plasma cells, occasional megaloblasts and normoblasts, small and large lymphocytes and large endothelial leukocytes. Polymorphonuclear leukocytes and myelocytes were almost completely absent; only one eosinophilic neutrophil was seen in the entire section. Occasional large mononuclear cells contained mitotic figures.

In the lower lobe of the left lung, thrombosis of some of the veins were seen; there was evidence of organization in thrombi, and surrounding them were regions

of infection. In scattered situations elsewhere the pulmonary alveoli were filled with fibrin and erythrocytes, but leukocytic infiltration was absent in the regions of consolidation. Sections of the right lung presented essentially the same appearance except for the absence of infection. In the rectum, a small region of necrosis beneath the intact mucosa was surrounded by a thin wall of lymphocytic infiltration, but there were no polymorphonuclear leukocytes.

Both kidneys revealed granular degeneration of cells of the tubular epithelium and small lesions of focal necrosis scattered throughout the cortex. These portions were infiltrated with lymphocytes and endothelial leukocytes. The wall of the prostatic abscess was edematous and infiltrated with lymphocytes. Polymorphonuclear leukocytes were entirely absent. Sections of the rectus abdominis muscle revealed small hemorrhages between bundles of muscle fibers and places in which the muscle fibers were swollen, hyaline in appearance, without striations and with completely degenerated nuclei. In the corium of the skin, there were miliary abscesses composed of small and large lymphocytes and a few polymorphonuclear leukocytes.

The anatomic diagnosis was paratyphoid fever, with multiple small ulcers in the colon; agranulocytosis; splenomegalia; hypertrophy of the liver; fatty changes in the myocardium; jaundice with hemorrhagic bullae and abscesses of the skin; bronchopneumonia; acute nephrosis; Zenker's degeneration of the rectus abdominis muscles, and multiple abscesses of the prostate gland.

Comment.—This case of hypogranulocytosis does not belong in the group of agranulocytic angina (Schultz's syndrome). It is of special interest because of the continued presence of *Salmonella schottmülleri* in the blood and the positive agglutinative property of the serum in high dilution for this organism. Whether there was an actual case of paratyphoid fever complicated with the prostatic abscess, or whether the prostatic abscess was primary and the septicemia secondary, must remain conjectural. The well marked anemia is also notable. The six transfusions of blood seemed of little value. Acriflavine was not given a fair trial.

Case 12.—A housewife, aged 55, came to the Mayo Clinic on April 14, 1930. The family history was essentially unimportant, and the patient had been healthy previously. Seventeen days before her registration at the clinic she had had so-called influenza, and her nose had been swollen. She had been given cathartics. Hemorrhoids, which she formerly had had, recurred, sloughed and left an anal ulcer. This caused soreness, and a feeling of anal obstruction and tenesmus.

At examination, an open wound was found, which involved the whole posterior portion of the anus. The concentration of hemoglobin was 52 per cent; erythrocytes numbered 3,870,000, and leukocytes 3,600 in each cubic millimeter of blood. A differential count was not made. Treatment of the ulcer and injection into the hemorrhoids was carried out, with good results. Thirteen days after rectal treatment there were general malaise, anorexia, aching in the bones, a temperature of 100 F., indefinite soreness in the left lower abdominal quadrant, headache and constipation. On the patient's readmission to the hospital the temperature was 101.5 F., and it varied from 100 to 104 F. until she died. At readmission, leukocytes numbered 350 in each cubic millimeter of blood, of which 51 per cent were lymphocytes and 36 per cent polymorphonuclears. Additional blood counts are given in the table. Attempts at agglutination of *Alcaligines abortus*, *Eber-*

thella typhi (Bacillus typhosus) and *Salmonella paratyphi (Bacillus paratyphosus A)* and *Salmonella schottmülleri (Bacillus paratyphosus B)* gave negative results. Blood cultures were contaminated. The concentration of bilirubin was 2.8 mg. in each 100 cc. of serum; the van den Bergh reaction was direct; the concentration of urea was 30 mg. in each 100 cc. of blood. Three days after admission there were soreness and a tender mass in the right lower abdominal quadrant. The day before death, ulceration developed on the right buttock, and there was a large hemorrhagic inflammation on the right arm. Operation for the abdominal abscess was not considered advisable. The concentration of urea was 126 mg. and that of creatinine, 5.2 mg. in each 100 cc. of blood on the day of death. The patient died eight days after she reentered the hospital. Permission for necropsy was refused.

Comment.—This patient did not have angina and cannot be considered as having had agranulocytic angina. The leukocytes were very few and the total number of granulocytes very small, although the percentage (35 per cent of 350) was not exceptionally low. There was leukopenia when the patient first entered the clinic, but former records of leukocyte counts were not available. This case illustrates, as does case 8, the possibility of the anus and rectum being the principal sites of the infection. There was moderate anemia.

CASE 13.—A contractor, aged 54, came to the Mayo Clinic on July 14, 1930. The family history was not significant. In April, 1930, the patient had become weak and fatigued, and boils had appeared on all parts of the body. His condition gradually had become worse. In the last six weeks before he came to the clinic the left leg and hand had been numb.

At examination, the temperature was 99.2 F.; there were slight icterus, many small furuncles, a palpable spleen and liver and a small anal ulcer. The concentration of hemoglobin was 18 per cent; the erythrocytes numbered 1,990,000; leukocytes, 1,000 and platelets, 144,000 in each cubic millimeter of blood. Reticulocytes were present in the proportion of 1.2 per cent. Additional counts are given in the tabulation. The Wassermann reaction was negative. The first blood culture was negative, but the second contained a streptococcus of a hemolyzing strain. The urine contained occasional erythrocytes and leukocytes. The concentration of bilirubin was 1 mg. in each 100 cc. of serum; that of urea was variously 32 and 38 mg. in each 100 cc. of blood, and the value for gastric acids was normal. The temperature ranged from 99.5 to 104.5 F. Treatment consisted of transfusions of blood and one intravenous dose of gentian violet. Death occurred thirteen days after the patient entered the hospital. The diagnosis was septicemia from a hemolyzing strain of streptococci, with hypogranulocytosis.

Comment.—This patient did not have angina. The hypogranulocytosis was associated with an infection of the skin and of the anus. There was marked anemia which was not of the aplastic type, as was indicated by the practically normal values for platelets and reticulocytes. The failure of the gentian violet given intravenously, and of the transfusions, to produce cure should be noted. The streptococcic septicemia probably was a secondary event.

CASE 14.—A hotel waiter, aged 40, came to the Mayo Clinic on Oct. 28, 1929. He had been well until March, 1929, when he had had chills, a temperature of 103 F. and urinary frequency. He had been in the hospital nine weeks, when he had had fever, dysuria, urinary frequency and pyuria. An abscess of the right foot had been incised, and pus had drained from it for six weeks. An abscess of the sternal region had been incised twice. Pain had developed in the lumbar portion of the spinal column, had increased and had been aggravated by motion. He had been practically bedfast since the onset of the illness and had lost 75 pounds (34 Kg.).

At examination there were a temperature of 99.5 F., slight redness of the throat, ulcerative gingivitis, a palpable spleen, the scar of an abscess on the right foot and a sinus in the sternal region. The concentration of hemoglobin was 63 per cent. The erythrocytes numbered 3,760,000 in each cubic millimeter of blood, and leukocytes, 2,000, 83 per cent of which were lymphocytes; 10 per cent, large mononuclears, and 7 per cent, transitionals; neutrophils were absent. Urinalysis disclosed a few pus cells and a trace of albumin in two specimens. The Wassermann reaction of the blood and blood cultures were negative. Smears and cultures from the mouth disclosed *Fusiformis dentium* and *Borrelia vincenti*, but no *Corynebacterium diphtheriae*. Two smears from the sternal sinus did not contain actinomyces or *Mycobacterium tuberculosis*. Roentgenograms of the thorax suggested that there were foreign bodies in the soft tissues below the left clavicle; those of the thoracic portion of the spinal column indicated that there was an increase in the density of the twelfth thoracic and the first lumbar vertebrae, suggesting metastatic carcinoma, whereas those of the kidneys, ureters and bladder did not give evidence of abnormality. The diagnosis was chronic infection with agranulocytosis, involving the urinary tract, spinal column, sternum and foot particularly. Tuberculosis was also considered. There was gradual failure, and the patient died twenty-three days after admission.

Necropsy revealed a deep ulcer over the right side of the manubrium and over the mesial third of the right clavicle. This ulcer represented the opening of a sinus between the second and third ribs on the right side, and behind the manubrium and the posterior surface of the upper half of the sternum, the tract also connected with a narrow channel that led to the right axilla. Along the latter tract there were several cystlike accumulations of a clear, light brown fluid. The spleen weighed 780 Gm. Its consistency was moderately increased. The lymphoid follicles were prominent. In the descending colon and rectum there were numerous, superficial, discrete, dark pink ulcers, more numerous in the lower part of the rectum. Between the twelfth thoracic and first lumbar vertebrae, an abscess was encountered which involved the intervertebral disk and which was continuous with a large cavity posterior to the right psoas muscle. The abscess contained 20 cc. of viscid, purulent material. There was no evidence of involvement of the vertebrae.

Sections of skin in the region of the ulcer presented little evidence of inflammatory reaction; there was practically no cellular infiltration. The sinus tract consisted of a thick layer of granulation tissue, which was infiltrated densely with lymphocytes, plasma cells and endothelial leukocytes, many of which were phagocytic. A part of the fibrocartilage of the intervertebral disk was replaced by fibrous tissue which was densely infiltrated with lymphocytes, plasma cells and many phagocytic cells, and there were numerous newly formed capillaries. The psoas muscle revealed only slight infiltration with lymphocytes and some degeneration of the muscle fibers.

The anatomic diagnosis was chronic spondylitis, with a paravertebral abscess on the left; chronic arthritis of the right sternoclavicular joint, with ulcer of the skin; ulcerative proctitis and sigmoiditis; splenomegalia, and agranulocytosis.

Blood Counts in Fourteen Cases

Case	Date	Time	Hemoglobin, per Cent (Dare)*	Cells in Each Cubic Millimeter of Blood: Erythrocytes, Millions	Cells in Each Cubic Millimeter of Blood: Leukocytes	Differential Cell Count of Blood, per Cent: Lymphocytes	Neutrophils	Eosinophils	Basophils	Monocytes: Large Mononuclears	Monocytes: Transitionals	Leukoblasts	Reticulocytes, Percentage of Erythrocytes in Same Fields of Smear	Platelets in Each Cubic Millimeter of Blood, Thousands
1	11/17/23	.....	75	4.27	12,700	31.0	64.5	1	0.5	1.0	2.0	...	...	...
	9/27/26	.....	79	4.36	10,000	.....	....	..	....	....	....	...	...	...
	1/ /27	.....	76	4.70	.....	.....	....	..	....	....	....	...	...	...
	2/23/28	.....	68	3.50	3,500	.....	....	..	....	....	....	...	...	...
	2/29/28	.....	77	4.07	6,400	.....	....	..	....	....	....	...	...	...
	3/29/28	.....	60	3.64	1,600	.....	....	..	....	....	....	...	...	...
	3/31/28	.....	..	....	1,600	96.0	....	..	....	....	....	...	...	...
	4/ 1/28	.....	..	....	1,700	.....	....	..	....	....	4.0	...	...	...
	4/ 2/28	.....	55	3.65	1,600	88.0	7.5	..	0.5	3.0	1.0	...	...	...
	4/ 3/28	.....	..	....	1,100	92.5	4.5	..	1.0	2.0	....	...	...	...
	4/ 6/28	.....	..	....	3,600	46.5	46.0	..	....	5.0	2.5	...	...	...
	4/14/28	.....	..	....	11,200	25.0	70.5	..	0.5	2.0	2.0	...	...	...
	4/16/28	.....	..	....	12,400	18.0	77.5	..	1.5	1.0	2.0	...	...	...
	4/25/28	.....	73	3.95	6,000	.....	....	..	....	....	....	...	...	...
	6/18/28	.....	76	4.01	2,800	77.0	23.0	..	....	....	....	...	...	...
	6/20/28	.....	..	....	2,800	.....	....	..	....	....	....	...	...	...
	6/21/28	.....	..	....	3,000	.....	....	..	....	....	....	...	...	...
	6/24/28	.....	..	....	1,500	98.5	1.5	..	....	....	....	...	...	...
	6/30/28†	.....	..	....	1,500	.....	....	..	....	....	....	...	...	...
	7/4/28	.....	50	3.46	1,000	.....	....	..	....	....	....	...	...	...
2	3/28/22	.....	64	4.68	7,800	.....	....	..	....	....	....	...	...	...
	12/19/24	.....	70	3.94	7,100	.....	....	..	....	....	....	...	...	...
	12/21/25	.....	59	4.03	7,000	.....	....	..	....	....	....	...	...	...
	1/12/26	.....	56	3.97	7,400	14.0	79.0	1	....	2.5	3.5	...	...	...
	12/ 7/26	.....	63	4.09	10,000	21.0	78.5	..	0.5	....	....	...	...	...
	11/16/28	.....	73	4.11	3,200	100.0	....	..	....	....	....	...	...	...
	11/17/28	.....	..	....	2,000	.....	....	..	....	....	....	...	...	...
	11/20/28	.....	..	....	1,100	100.0	....	..	....	....	....	...	...	...
	11/22/28	.....	..	....	1,100	100.0	....	..	....	....	....	...	...	...
		9:30 a. m.	..	....	900	.....	....	..	....	....	....	...	...	...
		12:30 p. m.	..	....	500	.....	....	..	....	....	....	...	...	...
		9:00 p. m.	..	....	200	.....	....	..	....	....	....	...	...	...
	11/23/28	7:30 a. m.	..	....	150	.....	....	..	....	....	....	...	...	...
		11:30 a. m.	45	3.34	150	.....	....	..	....	....	....	...	...	...
		3:15 p. m.	..	....	125	.....	....	..	....	....	....	...	...	...
	11/24/28	7:30 a. m.	..	....	100	.....	....	..	....	....	....	...	...	...
3	8/ 3/29‡	.....	66	3.40	1,000	89.0	11.0	..	....	....	....	...	...	...
	5/ 5/30‡	.....	..	....	2,800	.....	....	..	....	....	....	...	...	...
	5/12/30	.....	81	4.52	3,800	29.0	65.5	..	1.5	1.0	3.0	...	...	136
	5/13/30	.....	..	....	4,500	36.5	60.0	..	1.5	1.0	1.0	...	...	...
	5/20/30	.....	81	....	3,600	.....	....	..	....	....	....	...	...	...
	10/10/30	.....	77‡	4.88	2,400	69.0	29.0	..	1.0	1.0	.5	...	...	...
	11/20/30	.....	78‡	4.60	2,000	53.0	40.0	..	3.0	4.0	....	...	...	...
4	8/ 6/26	.....	..	....	10,700	.....	....	..	....	....	....	...	...	...
	8/22/26	.....	..	....	3,600	.....	....	..	....	....	....	...	...	...
	8/23/26	.....	..	....	3,100	.....	....	..	....	....	....	...	...	...
	8/24/26	.....	72	4.36	2,800	96.0	4.0	..	....	....	....	...	...	...
	8/25/26	.....	..	....	.....	.....	None	..	....	....	....	...	0.8	...
	8/26/26	.....	74	4.41	5,400	70.0	30.0	..	....	....	....	...	...	125
	8/27/26	.....	..	....	6,500	40.0	60.0	..	....	....	....	...	...	...
	8/28/26	.....	..	....	8,300	30.0	70.0	..	....	....	....	...	...	...
	8/30/26	.....	..	....	9,700	22.0	78.0	..	....	....	....	...	...	...
5	11/19/27§	.....	..	....	6,600	32.0	65.0	2	1.0	....	....	...	...	...
	5/ 3/28§	.....	..	....	6,700	32.0	63.0	3	1.0	....	....	...	...	...
	5/19/30§	.....	16.7 (Gm.)	....	8,700	.....	....	..	....	....	....	...	...	...
	6/25/30§	.....	60	4.17	9,600	.....	....	..	....	....	....	...	...	...
	7/ 2/30§	.....	..	....	8,500	.....	....	..	....	....	....	...	...	...
	12/22/30§	.....	13.1 (Gm.)	4.26	2,500	.....	....	..	....	....	....	...	...	...
	12/24/30	.....	..	....	1,400	84.0	2.0	..	....	14.0	....	...	...	118
	12/26/30	.....	..	....	5,700	28.0	46.5	..	....	25.5	....	...	...	...
	12/27/30	.....	..	....	6,800	47.0	51.0	..	....	2.0	....	...	...	...
	12/28/30	.....	..	....	3,400	50.0	45.0	..	....	5.0	....	...	...	...
	12/29/30	.....	..	....	4,200	39.0	55.0	..	....	6.0	....	...	...	...

Blood Counts in Fourteen Cases—Continued

Case	Date	Time	Hemoglobin, per Cent (Dare)	Cells in Each Cubic Millimeter of Blood: Erythrocytes, Millions	Cells in Each Cubic Millimeter of Blood: Leukocytes	Differential Cell Count of Blood, per Cent: Lymphocytes	Neutrophils	Eosinophils	Basophils	Monocytes: Large Mononuclears	Monocytes: Transitionals	Leukoblasts	Reticulocytes, Percentage of Erythrocytes in Same Fields of Smear	Platelets in Each Cubic Millimeter of Blood, Thousands
	12/30/30		..	....	7,000	40.0	57.0	..	1.0	2.0	....	...	...	...
	12/31/30		17.2 (Gm.)	4.77	5,150	31.5	64.5	..	1.0	3.0	....	...	...	...
	1/ 2/31		76	4.46	5,800	33.0	64.5	..	....	2.5	....	...	...	...
	1/ 3/31		..	....	7,150	30.0	67.0	..	....	3.0	....	...	...	...
	1/ 7/31		76	4.35	6,400	48.0	51.0	1	....	....	....	...	...	...
	1/10/31		..	....	6,100	49.0	49.0	1	1.0	....	....	...	...	...
	1/13/31		..	....	3,560	85.0	15.0	..	....	....	....	...	...	...
	1/14/31		..	....	2,930	71.0	27.0	..	....	....	2.0	...	...	...
	1/15/31		75	4.85	1,400	77.0	11.0	..	1.0	11.0	....	...	...	172
	1/16/31	a. m.	..	....	1,800	86.0	2.0	..	1.0	11.0	....	...	...	...
		p. m.	..	....	2,100	71.0	3.0	..	1.0	25.0	....	...	...	...
	1/17/31	a. m.	..	....	2,000	79.0	2.0	..	2.0	17.0	....	...	...	...
		p. m.	..	....	3,100	87.0	4.0	..	2.0	7.0	....	...	...	...
	1/18/31	a. m.	..	....	3,800	87.0	3.0	..	....	10.0	....	...	...	...
		p. m.	..	....	3,900	83.0	3.0	3	....	11.0	....	...	...	...
	1/19/31	a. m.	80	4.48	2,900	56.0	26.0	..	....	18.0	....	...	...	...
		p. m.	..	....	5,300	71.5	9.5	..	0.5	18.5	....	1.0	...	...
	1/20/31	a. m.	..	....	5,500	57.0	30.0	..	....	13.0	....	...	...	...
		p. m.	..	....	5,900	55.0	34.0	..	....	11.0	....	...	...	...
	1/21/31	a. m.	..	....	7,400	43.0	46.0	..	....	11.0	....	...	...	...
		p. m.	..	....	10,000	30.0	54.0	..	....	15.0	....	1.0	...	...
	1/22/31	a. m.	..	....	10,200	29.0	52.0	..	....	16.0	....	3.0	...	...
		p. m.	..	....	10,700	27.0	69.0	..	....	4.0	....	...	...	...
	1/23/31		..	....	10,900	27.0	64.0	..	2.0	7.0	....	...	...	...
	1/24/31		..	....	11,400	30.0	63.0	..	2.0	5.0	....	...	...	...
	1/25/31		..	....	11,600	.....	....	..	....	....	....	...	...	...
	1/26/31		..	....	12,000	19.0	75.0	..	....	6.0	....	...	...	...
	1/27/31		..	....	11,400	27.0	70.0	..	1.0	2.0	....	...	...	...
	1/28/31		..	....	12,700	28.0	72.0	..	....	....	....	...	...	...
6	8/20/21		72	....	7,700	.....	....	..	....	....	....	...	...	...
	7/ 5/22		70	....	8,400	.....	....	..	....	....	....	...	...	...
	2/28/27		71	4.59	.....	.....	....	..	....	....	....	...	...	...
	11/14/30		..	....	18,800	.....	....	..	....	....	....	...	...	...
	11/17/30		..	....	10,600	.....	....	..	....	....	....	...	...	...
	11/22/30		..	....	10,200	.....	....	..	....	....	....	...	...	...
	12/12/30		..	....	4,600	.....	....	..	....	....	....	...	...	...
	12/13/30		73	....	6,400	.....	....	..	....	....	....	...	...	...
	12/15/30		65	4.58	2,000	80.0	20.0	..	....	....	....	...	...	...
	12/16/30	a. m.	65	4.39	1,300	95.0	5.0	..	....	....	....	...	...	...
		p. m.	..	....	1,500	.....	None	..	....	....	....	...	...	...
	12/18/30	a. m.	55	3.55	1,000	.....	None	..	....	....	....	...	...	...
		p. m.	..	....	800	.....	....	..	....	....	....	...	...	...
	12/19/30	a. m.	55	3.46	800	.....	None	..	....	....	....	...	...	186
		p. m.	..	....	750	.....	....	..	....	....	....	...	...	...
	12/20/30	a. m.	50	3.35	800	.....	None	..	....	....	....	...	...	180
		p. m.	55	3.71	1,400	.....	....	..	....	....	....	...	0.2	140
				3.56	1,200	.....	None	..	....	....	....	...	...	154
	12/21/30		50	3.45	900	.....	None	..	....	....	....	...	...	144
7	3/11/30		..	4.24	6,100	94.0	....	..	....	6.0	....	...	...	...
	3/12/30		..	4.26	2,287	100.0	....	..	....	....	....	...	...	...
	3/13/30		..	....	1,100	100.0	....	..	....	....	....	...	...	...
	3/14/30		..	....	900	100.0	....	..	....	....	....	...	...	...
	3/15/30		85	4.76	950	100.0	....	..	....	....	....	...	...	...
	3/16/30		..	....	800	100.0	....	..	....	....	....	...	...	...
	3/17/30		..	....	650	100.0	....	..	....	....	....	...	...	...
	3/18/30		..	4.15	600	100.0	....	..	....	....	....	...	...	...
	3/19/30		..	....	3,950	13.0	76.0	..	....	11.0	....	...	...	...
	3/20/30		..	....	9,550	19.0	81.0	..	....	....	....	...	...	...
	3/21/30		..	....	7,600	14.0	86.0	..	....	....	....	...	...	...
8	12/20/29		10	0.86	4,000	76.0	18.0	..	2.0	....	4.0	...	...	...
	12/21/29		15	1.60	4,000	61.0	36.0	..	....	....	3.0	...	4.5	96
	12/23/29		38	2.64	3,800	49.0	49.0	..	....	2.0	....	...	3.0	128
	12/24/29		49	2.92	.....	.....	....	..	....	....	....	...	...	...

Blood Counts in Fourteen Cases—Continued

Case	Date	Time	Hemoglobin, per Cent (Dare)*	Cells in Each Cubic Millimeter of Blood		Differential Cell Count of Blood, per Cent							Reticulocytes, Percentage of Erythrocytes in Same Fields of Smear	Platelets in Each Cubic Millimeter of Blood, Thousands
				Erythrocytes, Millions	Leukocytes	Lymphocytes	Neutrophils	Eosinophils	Basophils	Monocytes: Large Mononuclears	Monocytes: Transitionals	Leukoblasts		
	12/26/29		52	3.18	4,000	36.0	61.0	..	....	....	3.0	...	...	...
	12/28/29		58	2.92	.....	....	....	..	....	....	....	...	...	...
	12/30/29		59	3.39	5,100	54.0	43.0	..	....	....	3.0	...	2.5	128
	1/ 2/30		51	3.00	4,000	51.0	46.0	..	....	....	3.0	...	3.0	96
	1/ 9/30		40	2.71	2,900	43.0	55.0	..	....	2.0	....	...	...	...
	1/18/30		38	3.02	2,000	73.0	26.0	..	....	....	1.0	...	...	...
	1/24/30		50	3.12	2,100	37.0	59.0	1	....	....	3.0	...	...	...
	1/27/30		52	3.13	4,300	20.0	78.0	..	....	....	2.0	...	...	...
	2/ 6/30		57	3.31	4,800	48.0	48.0	1	....	....	2.0	...	...	...
	2/19/30		58	3.00	4,000	69.0	26.0	..	....	3.0	2.0	...	...	...
	2/20/30		57	3.11	4,100	72.0	25.0	..	....	....	3.0	...	...	...
	2/23/30		45	2.25	1,000	84.0	14.0	..	....	....	2.0	...	...	...
	2/24/30		40	2.71	700	86.0	10.0	1	....	....	3.0	...	...	...
9	5/ /29		90	4.61	2,750	83.0	17.0	..	....	....	....	...	...	...
	7/26/29¶		80	4.74	2,300	76.0	11.0	..	....	3.5	9.5	...	...	...
			..	....	2,700	....	....	..	....	....	....	...	...	...
	7/29/29		61	4.51	2,300	75.0	8.5	..	....	5.5	11.0	...	...	242
			..	....	2,700	....	....	..	....	....	....	...	...	...
	6/20/30		..	....	....	48.5	45.5	..	....	1.5	4.5	...	...	...
	8/28/30		75	4.15	5,300	51.0	47.0	..	....	....	2.0	...	...	...
	10/13/30#		..	....	6,450	48.0	51.0	..	....	....	1.0	...	...	...
10	7/22/30		60	3.86	4,500	62.0	22.0	1	....	....	15.0	...	...	...
	7/24/30		70	4.09	2,700	39.0	51.5	..	....	4.0	5.5	...	3.3	...
	7/25/30		70	4.04	3,900	48.0	44.0	..	....	3.0	5.0	...	...	...
			..	....	4,400	....	....	..	....	....	....	...	...	...
	7/28/30		60	4.28	5,100	73.5	22.5	..	....	1.0	3.0	...	...	...
	7/29/30**		70	4.07	4,900	72.5	20.0	..	0.5	3.0	4.0	...	...	...
	7/30/30		70	4.13	4,000	67.0	22.0	..	1.0	3.0	7.0	...	...	...
11	10/ 3/29		30	1.77	2,200	84.0	13.0	1	....	2.0	....	...	...	100
	10/10/29††		30	2.08	1,000	78.0	21.0	..	....	....	1.0	...	1.7	80
	10/19/29		40	2.44	1,000	30.0	64.0	..	....	6.0	....	...	...	...
	10/27/29		..	....	1,800	44.0	56.0	..	....	....	....	...	...	...
	10/28/29		..	....	1,500	80.0	20.0	..	....	....	....	...	...	...
	10/30/29‡‡		..	....	1,600	....	....	..	....	....	....	...	...	...
12	4/15/30		52	3.87	3,600	....	....	..	....	....	....	...	...	...
	5/ 3/30		60	3.65	350	61.0	36.0	..	3.0	....	....	...	...	...
	5/ 6/30§§		..	....	1,300	41.5	51.5	..	5.5	1.5	....	...	...	60
	5/ 7/30		..	....	1,300	46.0	47.0	..	7.0	....	....	...	...	...
	5 /9/30		..	....	4,600	....	....	..	....	....	....	...	...	...
13	7/17/30		20	1.60	850	....	....	..	....	....	....	...	...	...
	7/18/30		15	1.66	750	94.0	6.0	..	....	....	....	...	1.2	82
	7/23/30		15	1.76	1,000	90.0	4.0	..	6.0	....	....	...	...	144
14	10/29/30		63	3.76	2,000	83.0	None	..	10.0	7.0	....	...	...	...
	10/31/30		..	....	3,500	32.0	62.0	..	....	6.0	....	...	...	...
	11/ 1/30		60	3.76	1,700	65.0	19.0	..	5.0	11.0	....	...	...	...
	11/ 4/30‖‖		..	....	3,200	56.0	37.0	1	....	6.0	....	...	...	...

* If hemoglobin is reported in grams for each 100 cc. of blood, this is indicated.
† Smears examined contained practically no granulocytes. Differential counts mislaid.
‡ Data furnished by Dr. George Parker, Peoria, Ill.
§ Data furnished by Dr. F. E. Vest and Dr. J. L. Lattimore, Topeka, Kan.
‖ One immature cell.
¶ Fragmentation of nucleus of neutrophils and irregular granulation seen in smears.
# Smears had normal appearance.
** In smears, marked evidence of toxicity in leukocytes; none of immaturity; good distribution of platelets.
†† In smears, evidence of toxicity graded 2 in leukocytes; none of immaturity.
‡‡ Additional leukocyte counts in text.
§§ In smears, evidence of toxicity graded 4 in leukocytes.
‖‖ In smears, nothing characteristic.

Comment.—Although the ulcerative gingivitis might place this case in the category of agranulocytic angina (Schultz's syndrome), it seems much more likely that the infection in the sternal and spinal regions and in the foot was a part of a generalized infection that was consequent on failure of the bone marrow, with its resultant leukopenia, or that the infection caused the leukopenia. The ulcerative gingivitis was probably only a local manifestation of diminished resistance to infection.

SUMMARY OF PATHOLOGIC STUDIES

The characteristic features noted in these cases were marked aplasia of the bone marrow and lack of the cellular reaction that is usually observed about regions of necrosis and infection. In the bone marrow, cells of the myelocytic series were barely to be seen, and proliferation of granular cells appeared to be at a standstill.

The peculiar inflammatory reaction that was observed seemed to be merely a consequence of the absence of circulating granular leukocytes. Tissue necrosis and ulceration, whenever they occurred, presented only the distinguishing feature of being unaccompanied by polymorphonuclear leukocytic infiltration. What cellular reaction did occur was comprised mainly of lymphocytic infiltration and the appearance of endothelial leukocytes (macrophage cells). Observation on the type of pathologic tissue reaction in this condition, namely, the lack of development of the protective inflammatory barrier to infection, suggested clearly the reason for the spread of such infection. It was evident, from complete pathologic studies in such cases, that the most common site for the development of focal regions of infection and necrosis was along various parts of the digestive tract, particularly at those points where bacteria are normally abundant. The involvement of the submaxillary gland in one of our cases might seem also to have been the result of extension of infection from the oral cavity. It is possible that these infections along the digestive tract were effected by the usual bacterial inhabitants which gained the ascendency as a result of the removal of the protective mechanism partially inherent in the normal function of the polymorphonuclear leukocytes.

The occurrence in the bone marrow of streptococci of hemolyzing strains in one of our cases probably had little bearing on the primary etiology of the disease. It seems more reasonable to accept the interpretation that there was metastatic invasion of the bone marrow through portals of entry established in various parts of the body, as, for example, through unprotected necrotic lesions in the digestive tract.

The pathologic features were essentially identical in so-called "agranulocytic angina" and in agranulocytosis, which, in one of our cases, was associated with paratyphoid fever. It seems most probable

that in one of our cases of agranulocytosis the original paratyphoid infection played a definite etiologic rôle, although one cannot, of course, exclude an associated, or secondary, independent infection which might have supervened to effect the pathologic process in the marrow; it may be that the infection was made possible by primary insufficiency of the bone marrow.

GENERAL COMMENT

The symptoms were diverse and the situation of the lesions was various. Seven cases presented a clinical syndrome that corresponded, in most details, with that described by Schultz, and three others probably belong in this group. The symptoms differed according to the character and intensity of the underlying disease, the situation of the principal lesions and the degree of resistance of the patient. Four presented agranulocytosis or hypogranulocytosis associated with infection manifesting itself outside of the throat. All displayed common reduction of the number of leukocytes and partial or complete disappearance of granulocytes.

In five of the fourteen cases there was a high percentage of monocytes (large mononuclears and transitionals) at some time in the course of the disease, and in another case now under observation, but not included in this report, the percentage of monocytes was 38. Three of these five patients recovered and the sixth, with 38 per cent monocytes, has made temporary recovery at least. On the other hand, two of the patients who died had increased percentages of monocytes. It may be that the increased percentage of monocytes indicates a relatively better prognosis than no increase, although a definite relationship cannot be seen in this group.

It is our opinion that the agranulocytosis and hypogranulocytosis probably are peculiar responses, in the bone marrow and the blood, to various inimical factors. No common etiologic factor appears. Blood cultures in five of our cases were negative. *Salmonella paratyphi, Klebsiella pneumoniae (Friedländer's bacillus)* and a streptococcus of a hemolyzing strain were found in one case each. In four cases, blood cultures were not made and in two there was a contamination. *Fusiformis dentium* and *Borrelia vincenti* were found in the throat in each of three cases; staphylococci, streptococci of a hemolyzing strain and green-producing streptococci were found in one case, and green-producing streptococci, staphylococci and a gram-positive bacillus in another case. In four cases there were no sore throat and no indication for examination of smears or cultures from the throat. In none was there a history of exposure to any of the chemicals or physical agents known to produce agranulocytosis. All presented evidence of an infection to which there seemed to be little resistance.

When the peculiar blood picture here described is found, a careful search for all possible causes should be made. Particular care should be given to the diagnosis of well recognized diseases that are associated with leukopenia and great reduction of granulocytes. Such diseases are aplastic anemia, acute leukemia with low values for leukocytes, pernicious anemia in an aplastic phase and metastasis to the bones producing the so-called myelophthisic anemia. Careful inquiry should be made regarding intoxication from benzene, the use of roentgen rays or radium and the administration of arsenicals, especially those of an arsphenamine group. Particular care should be paid also to cases in which there is marked increase in the proportion of lymphocytes and monocytes, with or without a decrease in the total number of granulocytes, such as occurs in infectious mononucleosis or glandular fever, chronic or acute lymphatic leukemia with low leukocytic values and monocytic leukemia. In cases in which there is hemorrhage, some difficulty undoubtedly may occur in eliminating from the diagnosis the various forms of purpura, especially purpura hemorrhagica. Moreover, acute aplastic anemia with hemorrhage, and acute leukemia with hemorrhage and a low total leukocyte count, must be excluded. Particular attention should be called to the value of the reticulocyte and platelet counts. The prognosis is extremely poor in any condition in which agranulocytosis develops; few patients have recovered if the leukocyte count has fallen below 1,000 in each cubic millimeter of blood.

Treatment has consisted of the administration of leukocytic extract, gentian violet and acriflavine, given intravenously; transfusions; roentgen radiation; extract of bone marrow, and fetal liver. In some cases specific treatment was not administered. The results with various methods of treatment were inconclusive.

SUMMARY AND CONCLUSIONS

Fourteen [11] cases are reported in which the outstanding feature was the leukopenia and partial disappearance of the granular leukocytes (hypogranulocytosis) or complete disappearance of them (agranulocytosis). In seven cases agranulocytic angina was definitely present, and in three it was doubtfully present. In the sense in which they are here used, the terms agranulocytosis and hypogranulocytosis do not indicate entities but probably stand for types of reaction of the leukopoietic apparatus to various types of infection or intoxication. Agranulocytosis is of serious prognostic significance. Death occurred in five of the seven cases of agranulocytic angina, in one of the three doubtful cases of agranulocytic angina and in all of the four cases of agranulocytosis or hypogranulocytosis due to other types of disease.

11. Patients in two additional cases not included in this group were under observation in the hospital at the time this report was being written.

CONGESTIVE HEART FAILURE

XII. THE RELATION BETWEEN THE THICKNESS OF THE CARDIAC MUSCLE FIBER AND THE OPTIMUM RATE OF THE HEART *

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The harmful effect of tachycardia in patients with cardiac disease is well recognized. A sustained increase in rate is not infrequently an important precipitating cause of a break in compensation. Occasionally one observes symptoms of congestive failure occurring during an attack of paroxysmal auricular tachycardia in a person whose heart is normal in every other respect. Such a case has been reported by Barcroft, Bock and Roughton.[1] However, some animals normally have heart rates which are considerably faster than those of patients with paroxysmal tachycardia. Why is a heart rate of 200 per minute likely to be attended by grave symptoms in men while the resting heart rates of guinea-pigs are more than 220? Again, why is it that the heart beats 600 times per minute in the mouse and 40 per minute in the horse (Clark [2])? If the optimum (i. e., the normal) heart rate is different in different species is it the same in enlarged hearts and hearts of normal size in the same species? What determines the optimum heart rate? It has occurred to us that the recovery time might be related to the length of time required for oxygen to diffuse into the fiber and that this might be greater for larger fibers.

The present researches were undertaken in an endeavor to throw light on these and similar questions in the hope that by so doing a better understanding of congestive heart failure might be attained.

METHOD

As a basis for the study of the normal heart rate electrocardiograms were taken on rats, guinea-pigs, rabbits, dogs, sheep and cows. Adult animals were used. At first a series of tracings were taken on a group of animals successively, but it was soon found that pulse rates obtained

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1. Barcroft, J.; Bock, A. V., and Roughton, F. J.: Heart **9**:7, 1921.

2. Clark, A. J.: Comparative Physiology of the Heart, New York, the Macmillan Company, 1927, p. 143.

in this way were much above the normal resting pulse rates. The factors of excitement, fear and struggling seemed responsible. Consequently, it was deemed wiser to restrict our series and search for individual animals that could be trained to become quiet while the tracings were made. It was necessary to take repeated tracings on several animals of each species in order to get one or two which were quiet and the pulse rates of which seemed relatively basal. In general, we have assumed that the presence of sinus arrhythmia indicated that the pulse rate was at, or nearly at, the truly normal resting level for the particular animal concerned. No narcotics or anesthetics were used. It was necessary to discard at least four fifths of our tracings, but it is felt that a few observations on quiet animals are more indicative of the normal pulse for the species than any number of unselected observations.

In order to determine the length of diastole the T-S intervals were measured. There is some question as to whether this interval corresponds exactly to the duration of mechanical diastole, but it seems justifiable to assume a fair agreement. The film speed and the standardization of the excursion of the string were varied in the different animals in order to obtain records which could be conveniently and accurately measured.

Electrocardiograms were also taken on six infants less than 6 months of age, on six children of from 3 to 6 years and on six children of from 9 to 12 years. Twelve electrocardiograms on normal adults were selected at random from the files. Similarly, electrocardiograms from six patients with cardiac enlargement without congestive failure, and from six patients with cardiac enlargement with congestive failure were selected. Measurements of the T-S interval were made in all instances.

In tabulating the data it was decided to use averages for the different human series and for dogs, sheep and cows, as the subjects of these species were quiet and seemed reasonably calm. However, since the rats, guinea-pigs and rabbits were usually restless, and even after considerable training many of them seemed excited, it was decided to discard all curves for these animals except those showing the slowest heart rates in each species.

Hearts were obtained from six animals of each species. Blocks of left ventricular tissue were cut at a level about half way between the base and the apex of the left ventricle. These were fixed, embedded, cut and then stained with hematoxylin and eosin. From necropsy material already available, six sections each were chosen of hearts of infants, children of from 3 to 6 years, children of from 9 to 12 years and adults. All these subjects had had normal hearts at autopsy. In addition, six sections were examined from the hearts of subjects who had, at autopsy, enlarged hearts without evidence of congestive failure, and six sections

were studied of the hearts of persons dying with clinical and pathologic evidence of congestive failure.

Measurements were made with the micrometer of the width of twelve muscle fibers of the left ventricle in each section, all measurements being made through the nucleus in order to be certain that comparable portions of the different cells were being studied. The greatest and the least values were then discarded and the average width of the other ten fibers was designated as the mean fiber thickness.

RESULTS

The Relation Between Fiber Thickness and Heart Rate in Human Subjects of Various Ages.—The data are shown in table 1 and charts

TABLE 1.—*Cardiac Fiber Thickness, Cardiac Rate and Length of Diastole in Human Subjects*

Condition of Heart	Age Group	Number of Cases Studied	Fiber Thickness, Microns			Pulse Rate per Minute			Length of Diastole, Seconds		
			Thinnest	Thickest	Mean	Fastest	Slowest	Mean	Shortest	Longest	Mean
Normal.....	Infants, 2 to 5 mo.	6	5.65	9.9	6.8	174	140	156	0.17	0.23	0.20
Normal.....	Young children, 3 to 6 yr.	6	9.37	11.7	10.4	135	100	118	0.24	0.36	0.29
Normal.....	Older children, 9 to 12 yr.	6	10.18	12.7	11.3	104	90	96	0.37	0.43	0.41
Normal.....	Adults	12	14.60	19.8	16.2	100	58	73	0.36	0.79	0.56
Enlarged; no congestive failure	Adults	6	18.80	27.0	24.5	115	60	84	0.30	0.68	0.49
Enlarged; congestive failure (digitalized)	Adults	6	26.50	42.7	31.8	85	60	76	0.43	0.64	0.53

1 and 2. It is evident that in subjects with normal hearts there is a definite inverse relationship between pulse rate and fiber thickness, and a direct proportionality between the length of diastole and fiber thickness. (We do not mean to imply that heart rate is "regulated" by fiber thickness.)

In patients with cardiac enlargement no such relationship exists. Their fibers are much thicker than those of the normal adult, but their heart rates are somewhat faster. (The average heart rate was more in those patients without, than in those with, congestive failure. This difference is probably to be attributed to the fact that the subjects in the latter group were digitalized.)

The Relationship Between Heart Rate and Fiber Thickness in Animals of Different Species.—That these two functions are in some way related appears from table 2, in which it can be seen that the animals with the fastest heart rates have the thinnest cardiac fibers and vice versa. In charts 3 and 4 it is shown that the relationship is not a

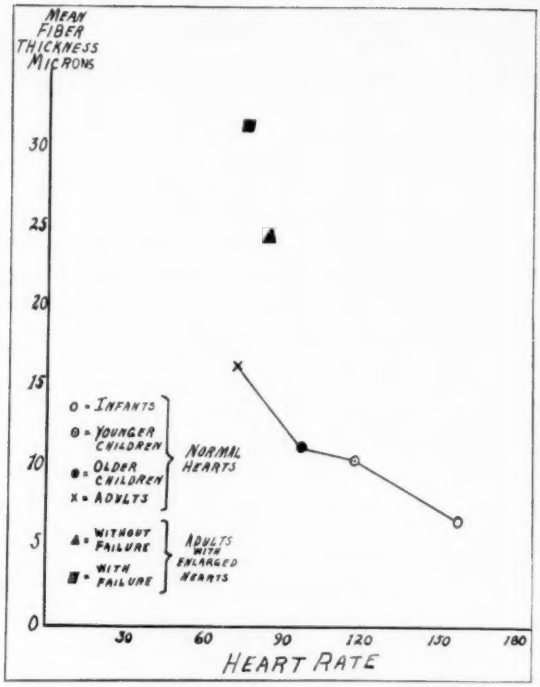

Chart 1.—An inverse and more or less linear relationship is shown between the heart rate and the fiber thickness of normal hearts. The values for enlarged hearts do not fall near the line.

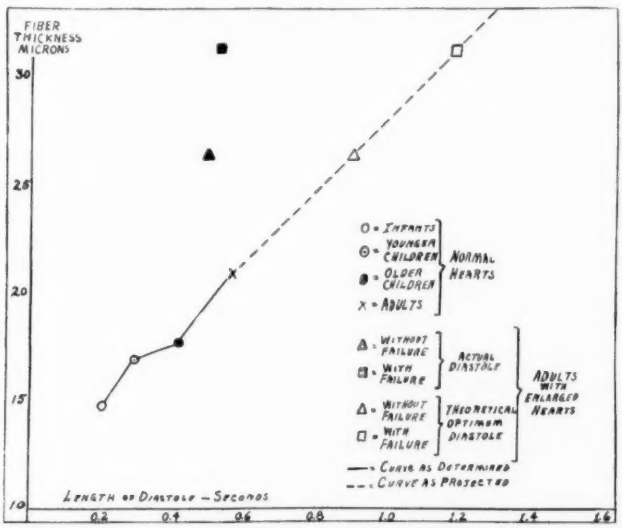

Chart 2.—Fiber thickness has been plotted against the length of diastole. The values for normal hearts in subjects of various ages fall reasonably close to a straight line. However, the points for enlarged hearts are not near this line. This seems to indicate that in such hearts the fiber is too thick for the rate, or the rate is too fast for the fiber thickness.

simple linear one but is suggestively logarithmic. In chart 5 the fiber thickness is plotted against the logarithm of the length of diastole, and the curve becomes a line. All the values for the normal animals fall reasonably close to this straight line—certainly as close as could be expected when one considers the numerous possible sources of error in the data. However, the values for enlarged hearts do not fall on the line at all, but are much above it. This is interpreted as meaning that their fibers are much too thick for their heart rates or, conversely, that their heart rates are much faster than optimal for their fiber thickness.

The observations on animal hearts are therefore in qualitative agreement with those on human hearts, and both indicate that a disproportion between heart rate and fiber thickness exists in patients with enlarged

TABLE 2.—*Cardiac Fiber Thickness, Cardiac Rate and Length of Diastole in Various Adult Animals*

Species	Number of Animals Studied	Mean Fiber Thickness, Microns	Pulse Rate per Minute	T-S Interval, Seconds
Rat	10	11.2	340*	0.10*
Guinea-pig	9	12.5	264*	0.15*
Rabbit	9	14.5	141*	0.28*
Sheep	6	14.8	105	0.40
Dog	6	16.8	69	0.56
Man	12	16.2	73	0.56
Cow	6	17.6	60	0.67

* The values given for fiber thickness are averages in all cases. The figures for pulse rates and T-S intervals are averages for those animals which remained quiet (man, cow, dog). The other animals usually either struggled or were excited, and the starred values were chosen from those animals which had the slowest pulse rates of their respective species.

hearts. From these data it seems fair to conclude that the optimum heart rate of patients with cardiac disease is considerably less than the optimum rate for normal hearts.

The next question is: How much slower than normal is this optimum rate? Our data furnish no satisfactory answer to the question. The two series of observations, although in qualitative agreement, do not agree quantitatively. The curve obtained from the animal hearts (charts 4 and 5) approaches the logarithmic form, whereas that obtained from the human hearts (chart 2) is nearly linear. When these curves are prolonged so as to reach the degree of fiber thickness of enlarged hearts, the former curve seems to indicate that the optimum rate for enlarged hearts is 10 or less, whereas the prolonged human curve—being linear—points toward an optimum rate nearer 40 for enlarged hearts. These points are illustrated in charts 2 and 5 and in table 3.

In view of this quantitative disagreement, which conclusion is one to believe? We think that the figures derived from the study of the animal hearts are more reliable, for two reasons.

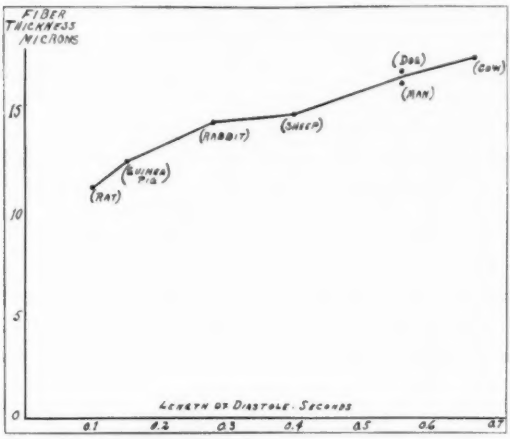

Chart 3.—Fiber thickness has been plotted against length of diastole for the normal hearts of the several species. An irregular curve has been obtained.

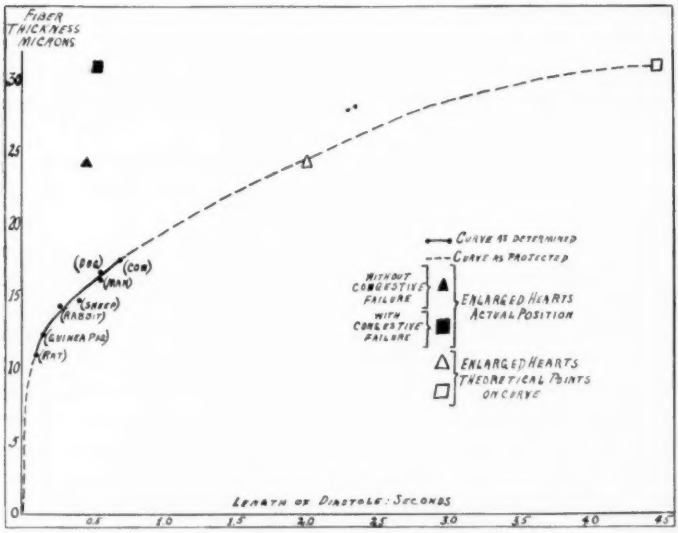

Chart 4.—The scale has been changed from that in figure 3. All the values for the normal hearts are fairly close to the curve, but the points for enlarged human hearts are much above it. When the curve is prolonged so as to attain the fiber thickness of the enlarged hearts, a rough estimate of the theoretical optimum length of diastole for such enlarged hearts can be obtained.

In the first place the animal hearts were from adults, and we are attempting to determine the optimum rate for enlarged adult hearts. It is possible that the factor of growth explains the obvious discrepancies between the two sets of data. Thus, the fiber thickness of the infant human hearts was only a little more than one-half that of the adult rat hearts, although the latter animals were less than one-fiftieth as large. Unless this difference is to be attributed to growth we are unable to explain it. In favor of the notion that growth is responsible is the fact that the human fibers are relatively thinner at both the age periods (infancy and 9 to 12 years) when growth is most rapid than at the age (3 to 6 years) when growth is less rapid.

In the second place a logarithmic type of curve, such as was obtained from the study of the animal hearts, is more in accordance with known laws of diffusion of gases. One can consider one half of the muscle

TABLE 3.—*The Calculated Optimum Heart Rate for Patients with Cardiac Hypertrophy*

Group	Mean Duration of Systole, Seconds	Calculations from "Animal Curve" (Chart 5)			Calculations from "Human Curve" (Chart 2)		
		Optimum Duration of Diastole, Seconds	Optimum Duration of Cycle, Seconds	Optimum Heart Rate per Minute	Optimum Duration of Diastole, Seconds	Optimum Duration of Cycle, Seconds	Optimum Heart Rate per Minute
Cardiac hypertrophy without congestive failure	0.22	5.89	6.11	10	0.90	1.12	54
Cardiac hypertrophy with congestive failure	0.26	50.2	50.28	1	1.19	1.45	41

fiber as being supplied with oxygen from the capillary on the corresponding side and the opposite half from the capillary on its side. The greatest diffusion distance is therefore one-half the thickness of the fiber, and the mean diffusion distance is one-fourth the thickness of the fiber. If the fiber thickness is doubled, the mean diffusion distance is doubled. However, the mean head of pressure (the oxygen tension in the capillary) is not doubled, but remains the same. As oxygen diffuses, the head of pressure diminishes. Hence, it probably diffuses more rapidly through the outermost than through the central portion of the fiber. Therefore, since the time required for complete recovery to take place after the contraction is dependent on the time required for oxygen to reach all parts of the fiber, one would expect than an increase of twofold in fiber thickness would more than double the recovery period. From such reasoning one arrives at the conclusion that the thicker the muscle fiber the slower the heart rate should be, not only actually but also relatively.

One objection may be raised to this concept, namely, that recovery might take place during systole as well as during diastole. Against this objection one may cite at least three points:

(*a*) If recovery can take place simultaneously with contraction there should be no limit, other than that set by mechanical factors, to the rate at which the heart or any skeletal muscle can contract, and this rate of contraction should be capable of being maintained indefinitely without fatigue. If this is true the normal human heart should be able to beat 250 times a minute for years.

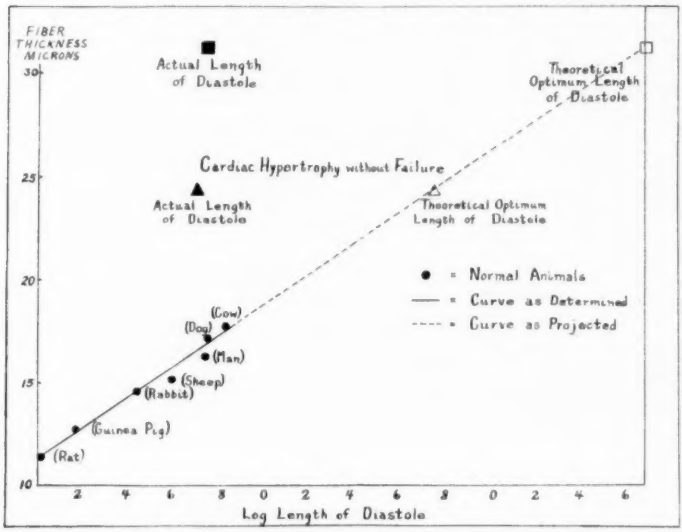

Chart 5.—Data on hearts showing hypertrophy with congestive failure. The fiber thickness is plotted against the logarithm of the length of diastole for hearts of the various species. The points so obtained lie very close to a straight line. This seems to indicate a logarithmic relation between fiber thickness and the length of diastole. Such a relationship is in agreement with A. V. Hill's calculations. The actual points for the enlarged hearts do not lie near the optimal normal line, but are much above it. By prolongation of the line, one can see that the optimum heart rate for hypertrophied hearts is apparently very slow.

(*b*) If recovery, a process which has been demonstrated to consume oxygen and to produce carbon dioxide, can take place during contraction, one ought to be able to demonstrate increased respiration in a muscle during active contraction. Moore [3] showed that the carbon dioxide production of tetanized frog's muscle was diminished during

3. Moore, A. R.: J. Gen. Physiol. **1**:613, 1918-1919.

the contracted state, even though this lasted for as long as thirty minutes. After relaxation a great increase in carbon dioxide production was observed. This experiment cannot be repeated on the heart because it cannot be tetanized, but there is no reason to believe that cardiac muscle differs from skeletal muscle in so fundamental a property.

(*c*) A. V. Hill [4] studied diffusion in thin strips of frog muscle suspended in oxygen. According to his calculations a strip of muscle 0.5 mm. thick, exposed to oxygen on one side at a temperature of 20 C., would be able to perform one twitch every eight seconds without acquiring an oxygen debt. If, all other conditions being the same, the muscle strip were twice as thick, only one twitch every forty seconds could be performed, whereas if the muscle were one-half as thick one contraction every two seconds would be possible. Hill stressed the importance of the size of the capillary, showing that the "time required for diffusion and recovery varies as the square of the average diameter of the vessels." He also said: "Mr. A. D. Ritchie has pointed out to me that an argument similar to that given here in relation to the size of blood capillaries may be applied to the case of muscle fibers; that is to say, the maximum diameter of a muscle fiber is probably not fixed by mechanical factors but by the speed of diffusion."

For these reasons we conclude that the optimum cardiac rate for patients with cardiac hypertrophy is probably in the neighborhood of 10 or less, the value derived from the study of the animal hearts, rather than about 45, which is the figure arrived at by the study of the human hearts. However, for practical purposes it is not important which figure is correct as both can only be crude approximations and as such a figure would naturally be less for a markedly hypertrophied than for a less enlarged heart. Both sets of observations agree in indicating that patients with cardiac disease, even when at rest and digitalized, still have rates which are much too fast.

The Frequency of Congestive Heart Failure in Patients with Heart Block.—If the conclusion which has been drawn—that the rate of the heart in patients with congestive heart failure is much faster than optimal—be correct, one would expect that persons with cardiac disease and abnormally slow heart rates would be less likely to suffer from symptoms of congestive failure than would persons with cardiac disease and normal rates. In order to study the matter, a number of cases of heart block were collected from the literature. No attempt at a complete survey was made.

The data are summarized in table 4. Edes [5] collected 121 cases of "permanent slow pulse" from the literature. His paper was written

4. Hill, A. V.: Proc. Roy. Soc. s. B., **104**:39, 1929.
5. Edes, T. R.: Tr. A. Am. Physicians **16**:521, 1901.

before electrocardiograms and polygrams were in general use, and there is some doubt as to whether all of these patients had heart block. However, most of them had pulse rates of 45 or less and many of them had convulsive seizures, so that there can be little doubt that most of Ede's cases showed heart block. In only one of the 121 cases was edema an outspoken symptom, and of the 10 patients with dyspnea at rest, 7 had it only during the convulsive seizures.

The reports by other authors were similar. A positive statement as to the absence of dyspnea and edema was not always made, but it is probably safe to assume that the symptoms would have been mentioned

TABLE 4.—*The Frequency of Symptoms of Congestive Heart Failure in Patients with Chronic Heart Block*

Author	Total Number of Cases Reported	Number of Cases in Which Edema Was Reported	Number of Cases in Which Dyspnea at Rest Was Reported	Comment
Edes [5]	121	1	10	Seven of the ten patients with dyspnea had it only during fainting spells. Diagnosis was not proved instrumentally, but most of the patients probably had heart block
Mackenzie (Diseases of the Heart, ed. 4, New York, Oxford University Press, 1925, p. 261)	18	1	1	
Parsonnet and Hyman (Am. J. M. Sc. **180**: 356, 1930)	8	1	1	
Hirschfelder [10]	3	0	0	
Blackhall-Morison (The Sensory and Motor Disorders of the Heart, ed. 2, New York, William Wood and Company, 1928, p. 241)	3	0	0	
Price and Nisse (Am. Heart J. **5**: 197, 1929)	2	0	0	Proved cases of heart block
Ascoli (Ztschr. f. exper. Path. u. Therap. **4**: 185, 1927)	1	0	0	
Harris [8]	1	0	0	
Willius [9]	1	0	0	
Total	158	3	12	

if they were at all prominent. Taking the case reports as given, one finds dyspnea at rest in only about 7 per cent and edema in only about 2 per cent of the cases. When one remembers that chronic heart block is most commonly found in patients with hypertension, arteriosclerosis, rheumatic infection and syphilis, and as a sequel to diphtheria (Butler and Levine [6]), and if one recalls that all of these diseases except diphtheria are common causes of congestive heart failure, the percentages seem remarkably low. The presence of heart block is proof that the underlying process has involved the heart and, a priori, one would have expected a much higher proportion of patients to have congestive failure, unless heart block in itself tends to protect against this.

6. Butler, S., and Levine, S. A.: Am. Heart J. **5**:292, 1930.

Against this argument certain objections may be raised:

(*a*) Butler and Levine have shown that a fairly large proportion of the cases of chronic heart block are presumably postdiphtheric, and diphtheria rarely causes chronic congestive failure. However, if we assume that diphtheria was the cause of the heart block in one half or even in two thirds of the cases collected in table 4, the percentage of cases with advanced congestive failure (edema) still would remain remarkably low.

(*b*) Dyspnea brought on by exertion is a common symptom in patients with chronic heart block. This is true, but symptoms brought on by exertion are to be attributed to diminished cardiac reserve and are not to be looked on as evidences of congestive failure. In patients with heart block the output per beat is very great even at rest (Lundsgaard [7]) and hence cannot be increased much, and, since their hearts do not accelerate during exercise, their dyspnea is probably due to an inability to increase the circulatory minute volume. Furthermore, many patients with heart block do not have dyspnea even with severe exertion (Harris,[8] Willius [9]).

(*c*) Perhaps patients with heart block do not live long enough for congestive failure to develop. On the contrary they often live for remarkably long periods after heart block has become manifest. Hirschfelder [10] reported a case in a patient with heart block of thirty-five years' duration. Mackenzie's and Harris' patients had had it for twenty-nine and twenty-eight years, respectively. None of these three patients had any evidence of congestive failure; all were leading normal lives. Most authors refer to the fact that complete heart block is compatible with long life and good health.

Furthermore, death in patients with heart block is usually sudden, and most frequently occurs during an attack of angina pectoris, or during a fainting spell. The slow agonal death with congestive heart failure is rare in such cases.

(*d*) Patients may develop heart block and congestive failure simultaneously. This is true, but it occurs only following overwhelming and sudden injury to the heart, such as occurs in certain cases of diphtheritic myocarditis and in patients with coronary occlusion. Such cases are acute and have been purposely omitted from table 4, which includes only cases of chronic heart block.

(*e*) Perhaps patients with heart block do not have cardiac hypertrophy. On the contrary, most of them do. Mackenzie, for example,

7. Lundsgaard, C.: Deutsches Arch. f. klin. Med. **120**:481, 1916.
8. Harris, E. H.: Heart **14**:289, 1929.
9. Willius, F. A.: Ann. Clin. Med. **3**:129, 1924.
10. Hirschfelder, A. D.: Diseases of the Heart and Aorta, ed. 2, Philadelphia, J. B. Lippincott Company, 1913, p. 571.

made a positive statement concerning the cardiac size in nine of the cases of his series with chronic heart block. In one case no enlargement was noted; in the other cases enlargement of varying degrees was found.

It therefore seems justifiable to conclude that heart block tends, other things being equal, to prevent congestive failure. We believe that the reason for this lies in the fact that such patients have heart rates which approach the optimal rate for hyperthrophied cardiac muscle fibers.

COMMENT

The thesis that congestive cardiac failure, regardless of the underlying etiologic factor, is immediately due to inefficiency (more commonly spoken of as insufficiency) of the cardiac muscle seems to be generally accepted. No one has yet offered an entirely adequate explanation as to why and how the myocardium fails. Attempts to explain the altered function on the bases of structural changes have been unconvincing. It is true that the majority of such hearts do show some fibrosis and some of them exhibit this change to an extreme degree, but in many instances the pathologic changes in the myocardium are altogether inadequate to account for its failure. This point of view has been particularly emphasized by Aschoff and Tawara,[11] and more recently by Christian [12] and by Kutschera-Aichbergen,[13] in whose paper a review of the literature may be found.

If myocardial failure cannot be attributed to myocardial disease, to what can it be attributed? The term "fatigue" has been applied to this gap in our knowledge, but a name is not an explanation. Overwork of the heart, which is usually present in such cases, is undoubtedly a potent factor, but overwork does not explain the progressive nature of the malady in the face of a load which is not increasing and is often diminishing. Progressive valvular or myocardial damage is more often absent than present in a large series of cases. Certain chemical changes in the cardiac muscle have been found, namely, alteration in the phosphatides by Kutschera-Aichbergen,[13] diminution in phosphates by Laszlo [14] and decreased potassium content by Harrison, Pilcher and Ewing [15] and by Calhoun, Cullen, Clark and Harrison.[16] However, there is, as yet, no convincing proof that such chemical changes are

11. Aschoff, L., and Tawara, S.: Die heutige Lehre von den pathologisch-anatomischen Grundlagen der Herzschwäche, Jena, 1906.

12. Christian, H. A.: South. M. J. **20**:28, 1927.

13. Kutschera-Aichbergen, H.: Wien. Arch. f. inn. Med. **18**:209, 1929.

14. Laszlo, D.: Klin. Wchnschr. **7**:1411, 1928.

15. Harrison, T. R.; Pilcher, C., and Ewing, G.: J. Clin. Investigation **8**:325, 1930.

16. Calhoun, J. A.; Cullen, G. E.; Clark, G., and Harrison, T. R.: J. Clin. Investigation **9**:693, 1931.

the sole or even the chief causes of fatigue, although it seems likely that they are in some way related to it.

The one outstanding clinical finding in most persons with chronic cardiac disease of various types is enlargement, and similarly the only pathologic changes which are almost invariably present in patients dying of chronic cardiac failure are hypertrophy and dilatation. It is believed that the observations which have been reported in this paper throw some light on the relation of cardiac failure to dilatation and hypertrophy. The studies of Eyster [17] suggest that dilatation, which is the immediate response to increased work (Patterson, Piper and Starling [18]), is the cause of hypertrophy, which is generally assumed to be the chronic response to overwork. Some authors hold that hypertrophy in itself constitutes disease. To this point of view we cannot accede, as the evidence for its being a compensatory process seems to us convincing. However, our observations seem to indicate that this compensatory process carries with it a disadvantage, the larger muscle fiber being too thick to admit of the ready diffusion of the necessary oxygen. Fatigue of muscle is generally believed to be due to lack of sufficient oxygen, and the observations which have been reported suggest the conclusion that the increase in fiber thickness is probably an important cause, although by no means the sole cause, of cardiac fatigue.

If this view is correct, it follows that a heart rate much slower than normal would be of great benefit to patients with hypertrophied hearts. This, of course, is merely a hypothesis based on deduction, and its truth or falsity can be demonstrated only by direct evidence. Attempts to secure such evidence are in progress.

SUMMARY

1. A study of ventricular fiber thickness, heart rate and the length of diastole has been made in a number of animals of different species, in normal human subjects of various ages and in human adults with enlarged hearts.

2. In all normal subjects a thick ventricular fiber is associated with a slow heart rate and a thin heart fiber with a fast heart rate.

3. It is believed that the slow heart rate in animals with thick cardiac fibers is advantageous because the recovery period of the heart is prolonged: i. e., it takes oxygen longer to diffuse through a thick fiber than through a thin fiber.

4. Subjects with enlarged hearts have heart rates much faster than would seem to be optimal for such thick fibers.

17. Eyster, J. A. E.: Tr. A. Am. Physicians **42**:15, 1927.

18. Patterson, S. W.; Piper, H., and Starling, E. H.: J. Physiol. **48**:465, 1914.

5. These observations suggest that "cardiac fatigue" may be due, in part, to a heart rate which is faster than optimal. It is believed that this concept may explain why congestive failure may occur in the absence of any myocardial abnormality other than hypertrophy and dilatation.

6. The data suggest further that if it were possible to reduce the cardiac rate to a level considerably below the normal, "cardiac fatigue" would be much benefited.

SEVERE RENAL INSUFFICIENCY

UNTOWARD EFFECTS OF INTRAVENOUS ADMINISTRATION OF SOLUTION OF SODIUM CHLORIDE *

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AND
NORMAN M. KEITH, M.D.
ROCHESTER, MINN.

Two cases are reported herewith of marked renal insufficiency with azotemia and without edema in which the administration intravenously of sodium chloride appeared to produce untoward effects. When the patients came under our observation, it was clearly indicated that if there was anything to be accomplished, it must be done by the intravenous administration of fluids. So far as was known, there were no definite contraindications to administering sodium chloride other than a suspicion that it might do harm. In these cases, the observations were not planned, but they were controlled accurately enough to permit of interpretation.

REPORT OF CASES

CASE 1.—A woman, aged 28, a teacher, came to the clinic in September, 1929, complaining of headaches and vomiting. She had enjoyed good health until five months before admission, when she began to have frontal headaches which came on while she was at work. Soon these headaches were accompanied by nausea and later by vomiting. The attacks at first were irregular, and she continued her work until about two and a half months before admission, when the attacks came so frequently that she became incapacitated. She had lost 30 pounds (13.6 Kg.) and was very sick.

The patient was pale (hemoglobin, 55 per cent, Dare), weak and low in spirits. There was an odor of urine on the breath. The lungs were clear; the heart was slightly enlarged, and the aortic second sound was definitely accentuated. The systolic blood pressure was 165, and the diastolic, 120 mm. of mercury. There was an irregular mass in the region of the right kidney. The reflexes were equal, and there was no edema. The pupils reacted normally. The ocular fundi were pale; the vessels were reduced in caliber, and there was choroiditis in the left eye. Urinalysis showed a trace of albumin and a specific gravity of 1.010.

When the patient was admitted to the hospital, she appeared lethargic, anemic and dehydrated. She weighed 47.2 Kg. (chart 1). Renal insufficiency was marked. No phenolsulphonphthalein was excreted in one hour. The blood urea content was 290 mg., and the creatinine, 12 mg. for each hundred cubic centimeters, and the effects of vomiting and dehydration were marked. For these reasons the immediate

* Submitted for publication, March 9, 1931.
* From the Division of Medicine, the Mayo Clinic.
* Read before the Central Society for Clinical Research, Chicago, Nov. 21, 1930.

administration of fluid in large amounts was indicated. Food and fluid taken by mouth were soon vomited. Accordingly, intravenous medication was immediately begun, 1,000 cc. of 10 per cent dextrose in 1 per cent sodium chloride solution being given during the first day, and 2,000 cc. of 10 per cent dextrose and 1 per cent sodium chloride the second day. In the next five days, from 1 to 2 liters of 10 per cent dextrose was given intravenously daily. The volume of urine gradually increased to 2,400 cc., the patient's weight decreased to 44 Kg., and the blood urea fell to 120 mg. From the eighth to the twelfth day, the fluid administered intravenously contained 1 per cent sodium chloride in addition to 10 per cent dextrose. Twelve days after admission, the patient's weight had increased to 48 Kg., and there was marked clinical improvement. The blood urea was 105 mg. for each hundred cubic

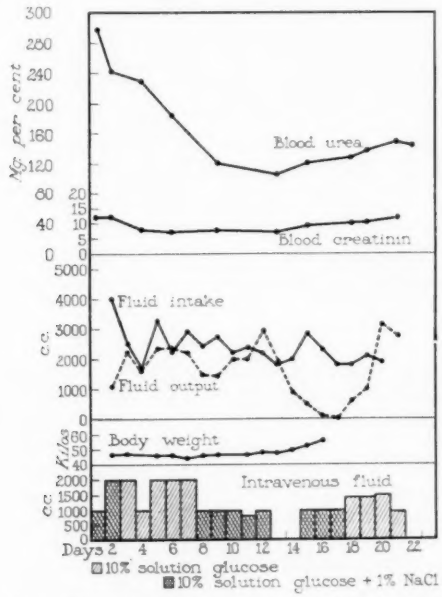

Chart 1.—Various effects of the intravenous administration of fluid in case 1.

centimeters. For the next two days fluid was not given by vein, and the volume of urine decreased to 900 cc. Intravenous medication was again instituted, and in the following three days the solution contained 10 per cent dextrose and 1 per cent sodium chloride. Oliguria developed and was followed by anuria, lasting for thirty-six hours. The patient's weight had increased to 55 Kg., and edema was noticeable in the face and skin of the lower part of the trunk. The urea and creatinine also increased in the blood. Because of the possibility of the sodium chloride causing the anuria and edema, it was discontinued during the next four days, the intravenous solution containing only 10 per cent dextrose. The excretion of urine was 100 cc. during the first day after the appearance of anuria and the total output of fluid was 600 cc., which included 500 cc. of vomitus. The volume of urine increased gradually to 2,600 cc. on the twentieth day of observation. Even with the restora-

tion of diuresis there was little change in the level of blood urea and of creatinine. During the entire three weeks the patient was in the hospital, urinalysis made as a routine disclosed just a trace of albumin and a specific gravity varying from 1.003 to 1.015, although the daily variation was usually from 1.005 to 1.009. On the twenty-second day of observation, the patient left the hospital. The subsequent course was practically unchanged; for one month, the headache, drowsiness, vomiting and oliguria persisted; then she began to improve slowly. The following April she felt so much better that she considered taking up regular office work. A year after her admission to the clinic, no subjective symptoms were apparent, although her physician was treating her for anemia. Our diagnosis was chronic glomerulonephritis with acute exacerbation and enlargement of the right kidney; the lesion in the latter was not determined.

Case 2.—A man, aged 37, a life insurance agent, had enjoyed good health until the onset of the present illness six weeks before admission to the clinic, on June 13,

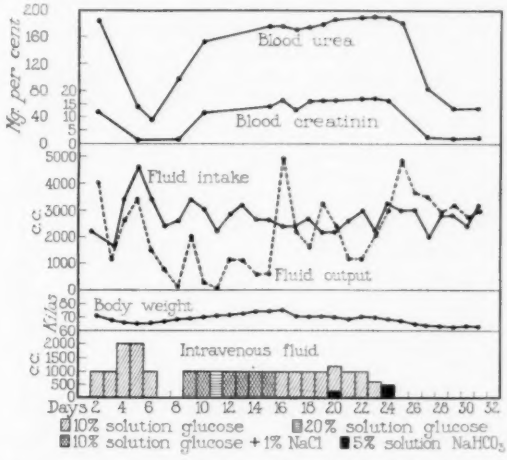

Chart 2.—Various effects of the intravenous administration of fluid in case 2.

1930. During the first week of May he had some backache and swelling of the right testis. He received four treatments of prostatic massage, which he thought made him feel better. Since January, 1930, he had had nocturia occasionally, which had become worse since the onset of the present illness. During the last two weeks before admission, he had frequency, but no dysuria.

The patient was somewhat anemic (hemoglobin, 50 per cent, Dare), but was well developed and well nourished. The odor of urine on his breath was noted. He did not appear to be critically ill. The tonsils were small, the lungs were clear, the heart was not large, the sounds were regular, and there were no significant murmurs. The systolic blood pressure was 195, and the diastolic, 105, and there was definite peripheral sclerosis. Nothing unusual was found in the genitalia. There was no edema, and the reflexes were normal. The pupils reacted normally. The ocular fundi were essentially normal. Preliminary studies showed that severe renal insufficiency was present, and on June 16 the patient was sent to the hospital for further study.

The striking feature presented by this patient on admission to the hospital was the evidence of serious renal insufficiency with few untoward symptoms. On the second day under observation (chart 2), the blood urea and creatinine were 184 and 12.4 mg. for each hundred cubic centimeters, respectively. Only a trace of phenolsulphonphthalein was excreted in the urine in one hour. Urinalysis showed a specific gravity of 1.011, only a trace of albumin and a few erythrocytes and leukocytes. The patient weighed 71 Kg. Treatment consisted of a diet containing from 1,500 to 2,000 calories, 40 Gm. of protein, from 2 to 4 Gm. of sodium chloride and from 600 to 2,600 cc. of fluid daily. An intravenous injection of from 1,000 to 2,000 cc. of a 10 per cent solution of dextrose was given daily. During the first three days, the output of urine was large, in the second period of twenty-four hours amounting to 4,000 cc. On the fourth day, the patient's weight was reduced to 65.7 Kg., and on the fifth day, the content of urea and creatinine in the blood had decreased to 56 and 1.5 mg. for each hundred cubic centimeters, respectively. Intravenous medication was discontinued on the seventh and eighth days. The daily

*Studies of the Urine in Case 2**

Day of Observation	Specific Gravity	Volume, Cc.	Sodium Chloride		Creatinine		Urea Nitrogen		Ammonium Nitrogen		Total Nitrogen	
			Per Cent	Total, Gm.	Per Cent	Total, Gm.	Per Cent	Total, Gm.	Per Cent	Total, Gm.	Per Cent	Total, Gm.
25	1.013	4,775	0.09	4.29	0.063	2.98	0.21	10.03	0.011	0.53	0.27	12.90
26	1.018	3,650	0.05	1.83	0.105	3.83	0.47	17.15	0.010	0.37	0.56	20.44
27	1.015	3,500	0.04	1.40	0.073	2.56	0.38	13.30	0.026	0.91	0.45	15.75
28	1.012	2,875	0.01	0.29	0.049	1.41	0.31	8.91	0.024	0.69	0.35	10.06
29	1.005	3,050	0.01	0.31	0.040	1.22	0.26	7.93	0.021	0.64	0.30	9.15
30	1.007	2,650	0.01	0.26	0.053	1.41	0.29	7.68	0.020	0.53	0.38	10.07
31	1.008	3,000	0.02	0.60	0.044	1.32	0.31	9.30	0.038	1.14	0.37	11.10
32	1.008	2,250	0.04	0.90	0.062	1.40	0.35	7.87	0.021	0.48	0.41	9.22
33	1.005	3,000	0.02	0.60	0.047	1.41	0.25	7.50	0.013	0.39	0.30	9.00
34	1.006	2,700	0.01	0.27	0.052	1.40	0.27	7.29	0.011	0.30	0.33	8.91
35	1.006	2,700	0.03	0.81	0.052	1.40	0.25	6.75	0.011	0.30	0.33	8.91
36	1.007	3,150	0.04	1.26	0.045	1.40	0.22	6.93	0.007	0.22	0.29	9.13
37	1.007	3,150	0.03	0.95	0.045	1.40	0.21	6.61	0.009	0.28	0.25	7.87

* Albumin was found in the urine only in traces. In all specimens trichloracetic acid failed to show precipitation. The diet consisted of 40 Gm. of protein, 1,500 calories, containing from 2 to 3 Gm. of sodium chloride daily.

intake of water by mouth was 2,500 cc., but the volume of urine diminished and the patient's weight increased to 69 Kg. The blood urea rose to 98 mg. Because of recurring renal insufficiency, daily intravenous injections were again instituted, and were continued for the next seven days (the ninth to the fifteenth day). The solution given contained 1 per cent sodium chloride and from 10 to 20 per cent dextrose. The daily volume of urine decreased to 600 cc., and the patient's weight increased to 74.8 Kg. There was no clinical evidence of edema accompanying the retention of water, although there was a marked rise in the blood urea and creatinine to 176 and 16.4 mg., respectively. The only time vomiting occurred during the patient's illness was on the eleventh and twelfth days, although nausea was occasionally present. In spite of this degree of azotemia, the content of chloride and the carbon dioxide combining power of the plasma were within the normal range. The sodium chloride in the solution given intravenously was suspected of having a harmful effect. On the sixteenth day, the patient was given 1,000 cc. of 10 per cent dextrose solution intravenously, and the urinary output amounted to 4,900 cc. The diuresis continued, and the patient's weight fell rapidly to 70 Kg. Contrary to what usually occurs with such marked polyuria, there was no decrease in the azotemia, the blood urea and the creatinine remaining high. These phe-

nomena were similar to those seen on the twentieth and twenty-first days in case 1. There was at this time (twentieth and twenty-third days) a lowering of the alkali reserve of the plasma, the carbon dioxide combining power being 33 and 37 per cent by volume, respectively. Accordingly, on the twentieth and twenty-fourth days, respectively, 200 and 500 cc. of 5 per cent sodium bicarbonate solution was administered intravenously. Remarkable diuresis followed the second injection and was accompanied by general clinical improvement, absence of nausea and an increase in the ability to drink water. The weight of the patient steadily decreased, and the content of blood urea and creatinine diminished rapidly. Within a period of six days, the weight had fallen to 63.2 Kg., a loss of 5 Kg., the blood urea from 189 to 54 mg. and the creatinine from 16 to 1.9 mg. This marked decrease in the blood urea and creatinine was accompanied by a great increase of these substances in the urine (table).

During the next three weeks, the patient gained in strength steadily. He continued to drink from 2,000 to 3,400 cc. of water daily, and the amount of urine varied from 2,300 to 3,900 cc. With these evidences of marked improvement, renal insufficiency was still present, the excretion of phenolsulphonphthalein being 25 per cent in one hour, the blood urea, from 22 to 80 mg., and the creatinine, from 1.9 to 3.6 mg. Because of moderate secondary anemia, a transfusion of 500 cc. of blood was given. At the time of dismissal, the patient was feeling well and his weight was 64 Kg.; the systolic blood pressure was 155, and the diastolic, 90. He was advised to continue with a diet consisting of 3,000 calories; it included 40 Gm. of protein, from 2 to 3 Gm. of sodium chloride and from 2,500 to 3,000 cc. of water. He was also given ferric citrate for the secondary anemia. A diagnosis of chronic glomerulonephritis with acute exacerbation was made. This patient was seen again in November, 1930. He was doing well and had been at work. The blood urea was 44 mg., and the creatinine was normal.

COMMENT

The two cases presented are of practical significance because they illustrate possible untoward effects from the intravenous administration of too much sodium chloride. Both of these patients had severe renal insufficiency with azotemia and isosthenuria but no edema. The specific gravity of the urine in case 1 ranged frequently around 1.010, and in case 2 around 1.006. Zondek [1] and Volhard [2] have observed that the concentration of sodium chloride in different samples of isosthenuric urine is inversely proportional to the concentration of nitrogen. This observation has been confirmed by us during one period of observation in case 2 (table). Zondek's and also Volhard's studies were limited to the concentrations of chlorides and nitrogen in the urine without reference to the concentrations of these substances in the blood and possible retention in the body. The oliguria that followed the administration of sodium chloride and the increased diuresis after the administration of

1. Zondek, Hermann: Ueber die Funktion der hämorrhagischen Nierentzündung von Kriegsteilnehmern, Ztschr. f. klin. Med. **83**:185, 1916.

2. Volhard, Franz: Die doppelseitigen hämatogenen Nierenerkrankungen (Bright'sche Krankheit), Berlin, Julius Springer, 1918, p. 64.

sodium bicarbonate, seen in case 2, indicate the sensitiveness of this patient to different inorganic radicals.

Temporary marked renal insufficiency and dehydration may be present in chronic glomerulonephritis. Administration of large amounts of water may have a remarkable beneficial effect. The intravenous injection of fluid may, in certain cases, be imperative, but when the solution contains too much sodium chloride, oliguria and other evidences of increased renal failure may develop. The optimal intravenous solution used by us has been 10 per cent dextrose.

Book Reviews

Die Roentgendiagnostik des Verdauungskanals einschliesslich der Leber und der Gallenwege. By Priv. Doz. Dr. H. U. Albrecht, Oberarzt an der medizinischen Universitäts-Klinik, Frankfurt, a.-M. Price, 59 marks. Pp. 471, with 828 illustrations. Leipzig: Georg Thieme, 1931.

Last year the author published a monograph of seventy-two pages, "Das Ulcus Problem im Lichte moderner Röntgenforschung," which was favorably reviewed in the ARCHIVES (**48**:169 [July] 1931). The present work enlarges the monograph to include the entire digestive tract. The high degree of excellence is retained. The illustrations are numerous, well chosen, clear and beautifully reproduced. The text is concise and direct; the subject matter seems to be presented by a clinician rather than a roentgenologist. The bibliographies are quite complete.

The first thirty pages are devoted to the esophagus: its anatomy and physiology and the various types of pathologic changes, including foreign bodies, ulcer, diverticulum, varicose veins, benign and malignant strictures, idiopathic dilatation of the esophagus and diaphragmatic herniation. Two hundred and fifty pages are employed in a similarly detailed discussion of the stomach and duodenum. This section includes the work previously presented in monographic form, and in addition a splendid discussion of gastric syphilis, gastric carcinoma, duodenal diverticula and the stomach on which an operation has been performed. The roentgenographic findings in the small bowel and appendix are briefly, but adequately, considered, including Meckel's diverticulum. The author thinks that nonvisualization of the appendix is suggestive but not diagnostic, and that much more significance is to be attached to an appendix that is fixed and tender. The eighty pages devoted to the colon include a presentation of the anatomy, physiology and pharmacology of the large bowel, as well as a study of its pathology. The various methods of examination are described and the results beautifully pictured; the ordinary barium enema, the air inflation method of Fischer, the "Reliefbild" of Knothe and the use of thorium dioxydsol as attempted by Kalkbrenner. The liver is disposed of in a four page discussion of the shadows that are seen on straight films of the abdomen made with and without pneumoperitoneum. The latter procedure is described, but is not recommended because the results are dubious and because occasional deaths have been ascribed to it. A fifty page consideration of cholecystography concludes the book. The author uses both the oral and the intravenous methods of administering tetiothalein sodium (sodium tetraiodophenolphthalein); he attaches little significance to variations in the intensity of the shadow, considers the presence of a shadow prior to the use of tetiothalein sodium as more suggestive of disease than a faint shadow, and lists the following as contraindications to cholecystography: cardiac decompensation, severe anemias, cirrhosis of the liver, febrile icterus, exophthalmic goiter, diabetes and chronic nephritis.

The work is of the finest quality and is highly recommended.

Normale und pathologische Funktionen der Verdauungsorgane im Roentgenbild. By Dr. Rudolf Becker, Roentgeninstitut des Theresienkrankenhaus, Mannheim, and Dr. Albert Oppenheimer, Roentgenabteilung der Med. Universitäts-Poliklinik, Frankfurt, a.-M. Price, 18.75 marks. Pp. 138, with 255 illustrations. Leipzig: Georg Thieme, 1931.

In this monograph the authors present the results of a detailed study of the motor function of the digestive tract under normal and pathologic conditions. The work is unique in that it utilizes the roentgenologic method in an attack on the physiologic problems of gastro-enterology. It is instructive, stimulating and well worth reading.

The book opens with a brief consideration of the principles of intestinal motility, the types of movement seen and the relationship between motility, tonus and the movement of content. This is followed by a section on the act of swallowing, including its physiologic variations, such as the ability of many people to drink without swallowing. Organic and functional disturbances in the esophagus are adequately considered, cardiospasm and spasm of the esophagus being discussed in well illustrated detail.

The section on the stomach is well done, but it contains little new material. The physiology and pharmacology of the pylorus are discussed, and the conclusion is reached that there are no exact criteria for the diagnosis of pylorospasm as distinguished from simple failure of the pylorus to open.

The two sections devoted to the small intestine and colon are the best in the book. Emptying of the duodenal bulb is ascribed to an active contraction of the bulb running from the tip to the base in a retrograde fashion, the peristalsis being a mirror image of that in the antrum. The action of the ileocecal valve is described in detail, and the motility of the terminal ileum is likened to that of the gastric antrum. Organic lesions of the small bowel are considered, including tuberculosis, adhesions and acute intestinal obstruction. The authors note that in diarrhea the disturbed motility is to be found in the colon, and not in the small bowel, confirming the clinical observations of Strasburger. A careful study has been made of the manner in which barium is carried along through the colon and of the changes observed in diarrhea and in constipation. The normal appendix is said to be visualizable, under proper conditions, in about 90 per cent of cases.

A brief section devoted to the gallbladder presents evidence of active motility of the organ, and describes pathologic conditions in considerable detail.

Clinical Dietetics: A Textbook for Physicians, Students and Dietitians. By Harry Gauss, M.S., M.D., F.A.C.P., Instructor in Medicine, University of Colorado School of Medicine. Assisted by E. V. Gauss, B.A. Price, $8. Pp. 490. St. Louis: C. V. Mosby Company, 1931.

In his preface, the author states that the book is a presentation in detailed form of lectures on clinical dietetics that he has been delivering for several years. In spite of this, much of the material is given quite briefly. The author demonstrates that he is a student and philosopher; that he has been accustomed to presenting things in a simple form. He has made the subject of dietetics seem relatively easy and interesting. The chapter on diabetes is presented simply and well, but too briefly. The author has striven throughout for simplicity, which often has been accomplished at the expense of the actual facts. The subject matter follows such a definite outline that relatively unimportant things are frequently overemphasized. Case reports are made to fit into this outline often without adequate justification. This is especially true of the ones used as illustrations of derangements of the digestive secretions. Lists of diets are given generously and constitute the best feature of the book. One general criticism to be made is that too much emphasis is placed on the use of roughage in practically all of the diets.